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(54) Title: SOLID SUPPORTS CONTAINING SCINTILLANT

(57) Abstract

A support for a chemical or biological application having chemically reactive sites, the support formed from at least one scintillant monomer by polymerisation or copolymerisation of the monomer. The supports may be used for biological scintillation assays, but also find new uses in solid phase synthetic chemistry and combinatorial chemistry.

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SOLID SUPPORTS CONTAINING SCINTILLANT

The present invention relates to scintillant polymers.

Scintillant polymers have many applications. Examples are their uses as solid supports in chemical and biochemical applications and as solid supports for chemical and/or biological reactions. The uses of the scintillant polymers disclosed herein are not limited to the uses exemplified.

Solid supports for use in chemical and biochemical applications are well known. Solid supports may be formed from a polymeric material such as a resin and, in such cases, will have the overall structure of a polymer matrix. Polymer resins of the type described above are termed solid supports since they contain covalent cross-links between their constituent polymer chains and are, therefore, insoluble in all solvents. Solid supports may be porous. They may be in the form of solid beads of any desired diameter, for example in the form of microspheres typically between 5-500μm in diameter; films; or a surface layer disposed, for example, on a microtitre plate or multipin synthetic apparatus.

In particular the present invention relates to supports incorporating a chemical group that can scintillate, emitting visible or otherwise detectable radiation, so as to aid in monitoring molecular interactions in chemical or biological systems or the progress of chemical or biological reactions. The present invention also relates to a method

for synthesis of such solid supports and their use in biological and chemical assays and for the synthesis and deconvolution of solid phase combinatorial chemistry libraries.

Scintillation can be defined as a flash of light produced in a phosphor by an ionising particle such as a beta particle or a photon. The term phosphor can be further defined as a phosphorescent or fluorescent molecule and in the text of the present application the terms "scintillant molecules" and "scintillant moieties" will be used to define molecules that react in this way, or a molecule that has a functional group that reacts in this way, and "scintillation" will be taken to mean light produced by such a scintillant molecule.

The scintillation phenomenon is used in scintillation proximity assays (SPA-s), where the light emitted by the scintillant molecule is detected and quantified in an appropriate scintillation counter. The SPA may be used to determine whether or not two different molecules interact. For example, if a biological receptor molecule is attached to a support such as a SPA bead (a solid support that contains scintillant molecules) and then placed in an aqueous solution containing a radiolabelled molecule, there may be binding of the radiolabelled molecule to the SPA labelled biological receptor. If such a binding interaction occurs, the radiolabel is brought into close proximity with the scintillant molecules contained in the bead. Radioactivity produces ionising particles and the close proximity of the scintillant and ionising radiation results

in emission of light. Tritium is used routinely as the radiolabel and emits ionising radiation with a very short path length; for example in water the average path length is 1.5 μm. If the distance between the tritium and scintillant molecules is greater than 1.5 μm no significant scintillation will result. Consequently, if there is no binding interaction between the SPA labelled receptor molecule and tritiated molecule, the majority of the tritium remains too remote from the scintillant molecules to cause scintillation. Thus scintillation, or the amount thereof, can be used to determine the extent of binding between the scintillant labelled receptor molecule and radiolabelled molecule. Such SPA results are quantitative, the degree of scintillation being dependent on the extent of the binding interaction.

European Patent 0 154 734 describes an immediate ligand detection assay marketed by Amersham as the Scintillation Proximity Assay, and describes a process for integrating fluorescent molecules, including 2,5-diphenyloxazole, into support bodies such as cyanogen bromide activated Sepharose 4B beads. The pores within the Sepharose beads are impregnated with fluorescent molecules via a precipitation process. Specifically, a DMSO solution of the fluorescent molecule is added to the beads so that the pores within the beads become filled with the solution. Addition of water to this system results in the DMSO being washed away from the beads whilst the fluorescent molecules (which are insoluble in aqueous solvents) are precipitated within the pores of the beads. The beads are then coated with a biological

receptor molecule through either non-specific, non-covalent interactions or else through covalent bonds via the cyanogen bromide. Beads prepared in this manner are incubated with a radiolabelled ligand. The radiolabel is chosen so that it emits beta particles or auger electrons that have a short path length in water. If the receptor binds to the ligand a significant portion of the radioactivity is brought into close proximity with the fluorescent molecules within the pores of the beads, these become activated and emit light. The light emitted can be detected and quantified directly in an appropriate scintillation counter. Conversely, if the receptor does not bind the ligand the majority of the radioactivity remains too remote from the fluorescent molecules to cause significant amounts of light to be emitted.

A related Amersham patent (EP 0 650 396) describes an extension of the SPA, namely the Cytostar-T Scintillating Microplates. This approach utilises microtitre plates which incorporate scintillant molecules within the base of each well within the plate. The surfaces of the well bases are coated with whole cells. Aqueous solutions containing radiolabelled substances are added to each well. If the cell incorporates the radiolabelled substance, the radiolabel is brought into close proximity of the scintillant molecules within the base of the well and light is emitted. The process may be adapted to study loss of signal when radiolabelled compounds are released from the cells. The scintillating properties of the plates have also been used to develop an *in situ* assay for mRNA. Scintillant

containing microtitre plates are also available from NEN Dupont (known by the mark Flashplates).

The receptor molecule can only be attached to the surface of such known scintillant beads using aqueous solvent systems since addition of organic solvents to the bead would result in the fluorescent molecules being dissolved and removed from the pores within the beads. Where scintillant microplates are used, the plates themselves are constructed from chemically un-functionalised polymeric materials which incorporate scintillant and which are incompatible with the use of most organic solvents. This restriction to aqueous solvents limits the applications of these known scintillant beads and plates.

The scintillation proximity assays described above have been used to study many biological receptor/ligand interactions (PCT Application Nos WO/98/15814; WO/98/03654; WO/97/47750; WO/97/45745; WO/97/28281; WO/97/26332; WO 97/10502; WO/96/21,156; WO/96/ 15,258; WO/93/19175; WO/90/11524; European patent publication Nos EP 781,853; EP 656,422;EP 378,059;and US Patent Application No. 768,652). In each case the assay procedure is necessarily carried out in aqueous solvents to prevent leaching of the scintillant molecules into the surrounding solvent. Even in aqueous solvents, leaching from the scintillant containing beads has been reported (Bosworth, Towers; Nature; 341; 67 (1989)).

Yttrium silicate doped with rare earth elements is an

inorganic based scintillator, and has been used in beads which may be coated with biological receptor molecules and derivatised. Beads of this type are now used in many SPA based applications (Amersham Pharmacia Biotech Catalogue 1998). However, yttrium silicate is not used as a solid support for solid phase synthetic organic chemistry.

The use of presently available scintillation proximity assays in the study of biological receptor/ligand reactions is further restricted by the need for a radiolabelled ligand. If the SPA assay is to be used to screen a number of potential ligands for binding ability to a specific biological receptor, either each potential ligand must be available in radiolabelled form, or a known radiolabelled ligand must be already available for displacement studies. This is potentially very expensive and may be extremely difficult to achieve.

Further, not all receptor molecules may be attached to known SPA beads.

Presumably, because of these drawbacks and others, there are still only a relatively small number of biological receptor SPA assays commercially available (21 in the 1998 Amersham Pharmacia Biotech Catalogue).

Though there are hundreds of commercially available solid supports for use in solid phase synthetic chemistry and combinatorial chemistry, it is impossible to use known SPA supports (such as beads) in solid phase synthesis

applications, because their use with organic solvents will cause leaching of the molecules, as outlined above.

According to the present invention there is provided a support for a chemical application, the support comprising a polymer matrix with a scintillant moiety covalently bonded into the polymer matrix.

By chemical application, it will be appreciated that any chemical, biochemical, or biological application is meant. The supports may be used in any application where a conventional support might be used. The supports may be used in, for example, monitoring molecular interactions in chemical or biological systems, monitoring the progress of chemical or biological reactions, bioassays and the like.

The scintillant moiety forms an integral part of the matrix of the support, as opposed to being grafted or tagged onto the surface of the support as a layer. The scintillant moiety is preferably substantially uniformly distributed throughout the polymer matrix.

The amount of scintillant may be, for example, 5-10 mol %, though it could be higher. Much lower concentrations of scintillant also give efficient scintillation. The resulting beads have a relatively large number of scintillant moieties and are sensitive to low levels of radioactivity. There is a further advantage in that the significance of scintillant quenching events in the application of the supports is relatively low.

Supports according to the invention do not fall prone to leaching when used in organic solvents.

By polymeric matrix it is meant a large scale solid structure made of polymer chains, these polymer chains being made up of smaller chemical units called monomers. There may be cross-linking, to varying degrees, between polymer chains, but this cross-linking is not essential. The chains and cross-links are not necessarily in defined or regular positions; the arrangement of monomers or the overall structure is not necessarily regular, and no crystallinity is implied.

Preferably, the support of the present invention is formed by a polymerisation reaction. The polymerisation may be achieved by any conventional polymerisation method.

The supports may be used as supports for chemical reactions, in which case the polymer matrix will also have chemically reactive site(s). By chemical reaction, it will be appreciated that any chemical, biochemical, or biological reaction is meant; it will similarly be appreciated that the term "chemically reactive site" may be taken to mean a site capable of binding/reacting with a chemical, a biological or biochemical molecule. It will be appreciated by one skilled in the art that the present invention is not limited only to the purely chemical field and will find much use in associated biological, pharmaceutical and biochemical fields.

The chemically reactive site may be incorporated into the polymer matrix. The chemically reactive site may be present as an integral part of the polymer matrix. Preferably, the chemically reactive site may be distributed substantially uniformly throughout the polymer matrix. If a support is made by a polymerisation reaction, the chemically reactive site may be incorporated during a polymerisation reaction step. Prior to the polymerisation, the chemically reactive site may be disposed on a monomer ("the chemically functionalised monomer"). On polymerisation, the chemically functionalised monomer will copolymerise with any other monomer(s) and reagent(s) present and the polymer matrix will thus be formed with integrated chemically reactive sites. Sites incorporated in this way may be distributed substantially uniformly throughout the polymer matrix, or, if desired, the polymerisation reaction may be controlled so as to restrict/localise the distribution of chemically reactive sites to one area, for example the surface of the matrix. A chemically functionalised moiety may also be incorporated subsequent to support formation using conventional solid phase synthetic techniques and reagents. It will be appreciated that on a molecular scale, the surface of the support polymer matrix may include cavities, channels and pores which will increase the surface area of the matrix/support available as a reaction surface as this "internal" surface is the surface that is accessible to solvents. The chemically reactive site may be formed at the surface of the matrix as a layer in the sense that it is disposed over the full surface area, that is disposed over both the exposed surface and the "internal" surface: the

cavities, channels and pores. The chemically reactive sites may be formed as a discrete external layer over the surface of the support.

These chemically reactive sites enable molecules to be bound covalently to the support, in contrast to the support of the invention with no chemically reactive site, which can be coated with molecules only through non-specific non-covalent interactions such as hydrophilic/hydrophobic or electrostatic interactions. Chemically reactive supports may be used in receptor immobilisation, in solid phase synthesis and in combinatorial chemistry.

Preferably, further additives may be incorporated into the support. These may be incorporated by polymerisation or any other conventional reaction. The additives may be, for example, porogens and/or templating molecules as described more fully hereafter.

Preferably, the support is in the form of a bead. Various diameter beads may be formed, depending on the reaction conditions of the synthesis. The polymerisation process may synthesise an assortment of different size beads. These may then be collected and divided by sieving. The bead may be of any diameter. Preferably, the bead is of diameter in the range 0.5 μm to 1 cm. Typical diameters might be in the ranges 37-75 μm, 75-90 μm, 90-150 μm, 150-300 μm and 300-500 μm. Alternatively, the polymerisation may produce a uniform bead size. Beads can also be produced of a size adequate for a single bead assay. The scintillant beads may have

high levels of polymer cross linking within the matrix (>5% and typically 20%), or low levels of cross linking (<5%). The latter are termed gel type polymers.

The support may be disposed as a layer in a reaction vessel surface, such as a microtitre plate. For plastics materials including microtitre plates, the most preferable physical characteristics would be for the polymer to be highly crosslinked. Other possible applications are as films and multipin synthetic apparatus, and for these latter applications, gel type polymers and highly crosslinked macroporous plastics are desirable.

The supports of the invention may be used to examine the interactions between molecules which are either non-covalently or covalently linked to the surface of the support, and molecules free in a solution which contacts the support. The molecules free in solution must contain some form of activator to cause scintillation on said intermolecular interaction, for example a radiolabel. The supports may also be used to detect interactions between the surface of the solid support itself and molecules free in a solution which will come into contact with the support, the free molecules having an activator, for example a radiolabel, to cause scintillation on interaction with the support.

In further applications of the invention, supports of the invention containing chemically reactive sites may be used as solid supports in solid phase synthetic chemistry and

solid phase combinatorial chemistry.

In a further application of the invention, the chemical modification of a molecule covalently attached to a support according to the invention may be studied, provided that the said molecule contains a radioisotope or other label which may be used to activate or in any way alter the properties of the scintillant moieties.

In a further aspect of the invention, a support for a chemical or biological application is formed from a scintillant monomer (a monomer comprising a scintillant moiety). Examples of scintillant monomers are described below. The support may be formed by polymerisation of one or more scintillant monomer(s), or copolymerisation of one or more scintillant monomer(s) with at least one additional monomer.

The additional monomer may be a monomer which comprises a chemically functionalised site or a chemically reactive site - "a chemically functionalised monomer". Examples of chemically functionalised monomers are 4-chloromethylvinylbenzene and p-acetoxystyrene.

The additional monomer may be a monomer which increases the bulk volume of the polymer matrix formed in the polymerisation reaction. Although such monomers react to form polymers or copolymers, they will not show a high degree of chemical reactivity once (co)polymerisation has occurred, and they will be termed hereafter "inert

monomers". Examples of such monomers are styrene and 4-ethylvinylbenzene.

Preferably, the support is formed by copolymerisation of a scintillant monomer and a chemically functionalised monomer. The support may also be formed by copolymerisation of a scintillant monomer and more than one chemically functionalised monomer. Instead, a support may be formed by copolymerisation of a scintillant monomer and an inert monomer. The support may also be formed by copolymerisation of a scintillant monomer and more than one inert monomer. A further preference is for a support formed by copolymerisation of a scintillant monomer, a chemically reactive monomer, and an inert monomer. Any number of monomers (of any type) may be polymerised with at least one scintillant monomer to form a support according to the invention.

On polymerisation, the scintillant monomer will copolymerise with any other monomer(s) and reagent(s) present and the polymer matrix will thus be formed with integrated scintillant sites. Sites incorporated in this way may be distributed substantially uniformly throughout the polymer matrix, or, if desired, the polymerisation reaction may be controlled so as to restrict/localise the distribution of scintillant sites to one area, for example the surface of the matrix.

A cross linking agent may be used in the polymerisation. The cross linking agent may be a monomer. An example of a cross

linking agent is divinylbenzene. Increased cross linking will reduce the likelihood of the support dissolving in organic solvents. The polymerisation may be of the scintillant monomer only, but in this case it might be highly desirable to add a cross linking agent.

The scintillant monomer may be chemically functionalised to produce a support with chemically reactive sites. For example, any of the scintillant monomers described hereafter may be chemically functionalised by substituting a chemically functionalised group onto one (or both) phenyl groups of the diphenyloxazole moiety.

The scintillant monomers may be used to construct specifically shaped supports, or may be disposed as a layer on a support or on a reaction vessel surface.

According to the present invention in a further aspect, there is provided a scintillant monomer comprising a scintillant moiety and a separate polymerisable moiety. Preferably the separate polymerisable moiety includes an alkene group. Preferably these are distant from each other within the scintillant monomer, so as to prevent electron delocalisation within the scintillant moiety being disrupted by the polymerisation reaction. Any such disruption during polymerisation may have a detrimental effect on the scintillant activity of the support.

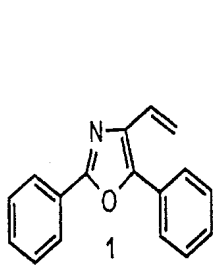
The scintillant monomer comprises a molecule of structure:

R-Y;

wherein R is a scintillant group; and Y is a group which includes a polymerisable moiety.

Preferably R is a 2,5-diphenyloxazole group. Preferably Y is a substituted or unsubstituted aliphatic or aromatic group; or an ether.

Preferably the scintillant monomer has one of the following structures (1) - (5)



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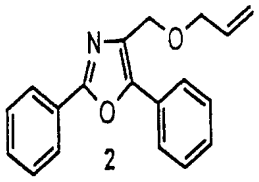

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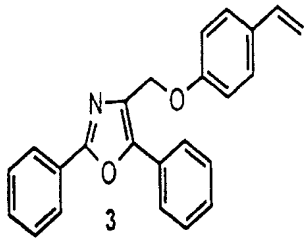

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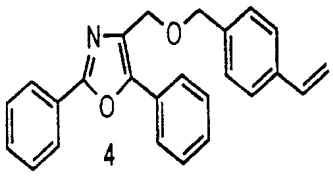

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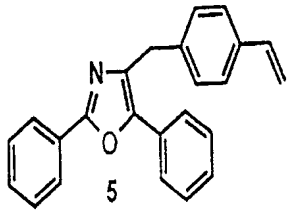

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According to the invention in a further aspect there is

provided a scintillant polymer formed by polymerisation or copolymerisation of at least one scintillant monomer. The scintillant monomer comprises a scintillant moiety and a separate polymerisable moiety. Preferably, the scintillant monomer is one of structures (1) to (5).

According to the present invention in a further aspect there is provided a method of preparing a scintillant polymer comprising the steps of [a] taking at least one scintillant monomer, and [b] effecting a polymerisation step.

The scintillant polymer may instead be formed by copolymerisation of a scintillant monomer and at least one additional monomer.

According to the present invention in a further aspect there is provided a method of preparing a scintillant polymer comprising the steps of [a] taking at least one scintillant monomer and at least one additional monomer, and [b] effecting a polymerisation step.

Any conventional polymerisation process may be used to produce the scintillant polymer: bulk, suspension, emulsion and solution reactions are all suitable.

Once polymerised or copolymerised, it will be appreciated that the scintillant monomer is incorporated into the polymer. In this specification, the term "scintillant monomer" may be taken to mean the scintillant monomer before it has been polymerised. The term "scintillant monomer" may

also be taken to mean that part of the scintillant monomer structure that is incorporated into the polymer or copolymer once the polymerisation step has taken place.

Preferably, the scintillant monomer is one of structures (1) to (5). The additional monomer may be a chemically functionalised monomer. The additional monomer may be a monomer that increases the bulk volume of the polymer matrix formed in the polymerisation reaction: "inert monomers", as described hereinbefore. Examples of chemically functionalised monomers are 4-chloromethylvinylbenzene and p-acetoxystyrene. Examples of inert monomers are styrene, 4-ethylvinylbenzene and divinylbenzene.

Scintillant monomers of the invention, and the methods of producing scintillant polymers and scintillant supports according to the invention, may be used to prepare a support for a chemical application or a chemical reaction.

The support may be in the form of a bead. The bead may be of diameter 0.5μm to 1cm. The support may be a gel-type polymer support. The support may have macroporous structure.

Any conventional polymerisation process may be used to produce the scintillant support: bulk, suspension, emulsion and solution polymerisation reactions are all suitable. Consequently, scintillant supports with a variety of physical properties and forms can be produced. Beads may be formed by a suspension co-polymerisation in which one of the monomers is a scintillant monomer.

Preferably, the support is formed by copolymerisation of a scintillant monomer and a chemically functionalised monomer. The support may also be formed by copolymerisation of a scintillant monomer and more than one chemically functionalised monomer. A support may instead be formed by copolymerisation of a scintillant monomer and an inert monomer. The support may also be formed by copolymerisation of a scintillant monomer and more than one inert monomer. A further preference is for a support formed by copolymerisation of a scintillant monomer, a chemically reactive monomer, and an inert monomer. Any number of monomers (of any type) may be polymerised with at least one scintillant monomer to form supports according to the invention.

Preferably, the scintillant monomer is one of structure (1) to (5). Examples of chemically functionalised monomers are 4-chloromethylvinylbenzene and p-acetoxystyrene. Examples of inert monomers are styrene, 4-ethylvinylbenzene and divinylbenzene.

The polymerisation reactions described above may be carried out either in the presence or absence of chemical cross-linking agents such as divinylbenzene. If no cross-linking agent is used, the resultant polymers may be soluble in organic solvents. However, if a cross-linking agent is used, the resultant cross-linked polymers contain covalent linkages between their constituent polymer chains. The degree of cross-linking in a solid support varies its properties considerably. A solid support with a low degree of cross-linking (typically <5%) may

swell considerably in some organic solvents (but not actually dissolve) and is termed a gel-type solid support. In contrast, highly cross-linked solid supports do not swell in organic solvents.

A porogen may sometimes be added to a polymerisation reaction. Examples of porogen molecules include toluene and 2-ethyl-1-hexanol. The porogen is chemically inert to the polymerisation reaction conditions and is used to introduce pores into the product polymer. After the polymerisation reaction, the porogen is easily removed from the product polymer, by, for example soxhlet extraction. Solid supports constructed in the presence of a porogen are said to be macroporous. The resultant polymers scintillate in the presence of ionising radiation.

A template molecule may be added to the polymerisation reaction. This is done to imprint locations/environments with known electronic and/or structural identity (that is, the identity of the template) into the support during the polymerisation process. Once polymerised, the template is incorporated into the support. The template may be bound into the support in a covalent manner or non-covalently. To leave the template locations of known electronic and/or structural identity, the template is removed. An example of a template molecule would be cholesterol. The template molecule may added as a free molecule, or as a derivative. If the template is added as a free molecule, it may be removed by washing. A derivative may comprise the template molecule and a polymerisable site. In the latter case, the template is covalently bound into the support, and will have to be removed by cleaving the template molecule

from the polymerisable site.

In a further application of the invention supports comprising scintillant monomers and chemically reactive sites may be used as solid supports in solid phase synthetic chemistry and solid phase combinatorial chemistry.

According to the invention in a still further aspect, there is provided an assay incorporating the steps of:

[a] providing a support for a chemical application comprising a polymer matrix having at least one scintillant moiety and at least one chemically reactive site, the scintillant moiety being covalently bonded into the polymer matrix;

[b] mixing the support with a molecule comprising an activating group and a site which may react with the reactive site on the support; and

[c] measuring the scintillation produced by the scintillant moiety.

The activating group may be, for example, an α emitter, β emitter, or an Auger electron emitter. Preferably the ionising group is a radiolabelled group, such as a tritiated group or a group labelled with ^{125}I, ^{35}S or ^{33}P.

Throughout the specification, mention is made of causing scintillation by the close proximity of a radiolabelled molecule. The present invention is not limited to the use of

radioisotope labelling; anything which activates the scintillant molecule or in any way alters its fluorescent or scintillant properties may be employed.
Preferably, the support is a bead. The assay may be performed using a single bead, or many beads.

The supports of the invention are particularly suitable for use in solid phase synthetic chemistry. Solid phase synthetic chemistry has been known for many years. Conventionally, a solid support is used as a support for a stepwise synthesis of a molecule. As a first step, a "base"-reactant molecule is covalently bound to the support at one position and remains so bound during each step of the synthesis. The desired reaction takes place at one or more chemically reactive location(s) elsewhere on the reactant molecule. This covalent attachment between the base molecule and the support means that after each successive chemical reaction, the reaction solvent, any unreacted reagents and any reaction by-products can be removed simply by washing the solid support with an appropriate organic solvent, while the reacted base molecule remains bound. All reagents can be employed in excess and thus all of the chemical reactions can be driven to completion.

Conventional solid supports include Merrifield's resin, Wang resin, Rink resin, Sieber resin and PEG polystyrene resin. These are not suitable for use with SPA techniques as they contain no scintillant moieties.

According to the invention in a still further aspect there is provided a support for use in solid phase synthetic chemistry

comprising a polymer matrix with at least one scintillant moiety covalently bonded into the polymer matrix, and at least one chemically reactive site.

It will be possible to attach virtually any molecule to these supports by links which are specific and covalent in nature.

Preferably the support is in the form of a bead. The bead may be of diameter 0.5μm to 1cm. The bead may be a gel type polymer. The bead may have macroporous structure. The structure may be highly crosslinked.

The synthetic chemistry may be performed using a single bead, or many beads.

The support may be a gel type polymer.

The supports of the invention may be used in combinatorial chemistry.

This is a branch of synthetic chemistry which centres on the simultaneous production of large numbers of structurally related compounds: "a library". The library may include positionally fixed components. The compounds within the library are screened simultaneously to determine if one or more compounds exhibits a desired property, for example, the ability to bind to a biological receptor molecule. If this is found to be the case, it is then necessary to identify the chemical structure of the 'active' compound(s). The process whereby the chemical structure of the active compound(s) is deduced is

termed deconvolution, and is the crux to any successful combinatorial chemistry strategy.

In Solid Phase Combinatorial Chemistry, library compounds are synthesised on the accessible surfaces of a chemically reactive solid support, such as a polymeric resin bead.

By far the most common solid phase combinatorial chemistry strategy employs resin beads as the solid support and uses a 'split and mix' method to synthesise the library compounds.

Figure 1A shows a schematic representation of the 'split and mix' method.

The 'split and mix' method also called the 'one bead, one compound' approach enables libraries that contain large numbers of compounds to be constructed extremely rapidly. After library synthesis, each resin bead bears multiple copies of the same library compound, with different beads bearing different library compounds. The library of compounds is then screened *en masse* for a desired property such as the ability to bind to a biological receptor.

In a typical conventional assay procedure, the beads bearing the library compounds are incubated with a dye labelled receptor molecule. Should a library compound bind to the biological receptor, the bead bearing that compound will appear more coloured than the other beads. Assuming this to be the case, the most intensely coloured bead is physically removed from the other beads.

It is then necessary to determine the identity of the 'active'

library compound attached to this bead. Two commonly used methods are i) positionally fixed library synthesis and ii) encoded library synthesis.

Figure 1B shows a schematic representation of positionally fixed library synthesis.

This approach, is widely used in solid phase combinatorial chemistry. A library of the type X-X-X-X shown in figure 1B, has each position within the library fully randomised and may be for example be one of six different amino acids (A-F). In addition to synthesising a single library where each position is fully randomised, four different sets of six 'positionally fixed' sublibraries are synthesised. In the first set of six sublibraries position 1 is fixed as each of the six amino acids in turn, whilst positions 2, 3 and 4 are fully randomised. In the second set, position 2 is fixed as each of the six amino acids in turn whilst positions 1, 3 and 4 are fully randomised. In the third set of six sublibraries, position 3 is fixed and in the final set, position 4 is fixed. All 24 sublibraries are then screened for a specific activity. Again, in a typical assay procedure, the beads bearing the library compounds are incubated with a dye labelled receptor molecule, and the assay completed as described above. The most active sublibrary within each set of six indicates the identity of the optimum amino acid at the fixed position. The identity of the optimum compound within the library may thus be deduced (in this case C-D-F-B).

In Encoded Library Synthesis, at each stage of library synthesis, a coding molecule is attached to the resin beads.

Once a bead bearing an active compound has been identified, again, typically by using a dye based assay, as described above, the coding molecule(s) attached to the bead are analysed to allow the code to be deciphered and thus enable the identity of the active compound to be determined. An alternative coding strategy utilises programmable memory devices which can be programmed and read remotely.

Scintillation assays as used in combinatorial chemistry strategies as outlined above are problematic.

Conventional SPA's cannot be used readily with positionally fixed library synthesis or encoded library synthesis, since, in either strategy, all of the solid phase synthetic chemistry steps utilised to construct the library compounds require the use of organic solvents. Conventional SPA beads and scintillant microplates are incompatible with solid phase synthetic chemistry as, for example, they are incompatible with the use of most organic solvents.

In order to use a conventional scintillation type assay with either positionally fixed library synthesis or encoded library synthesis the library compounds must be cleaved from the solid support into spatially addressable vessels. Additionally, each library compound itself has to be synthesised in radiolabelled form, (which can be expensive and difficult to accomplish), or a known radiolabelled substrate for the target receptor must be available or synthesised for displacement studies (which again can be expensive and difficult to accomplish).

According to the present invention in a further aspect there is provided a support for use in combinatorial chemistry comprising a polymer matrix with at least one scintillant moiety covalently bonded into the polymer matrix, and at least one biochemically/chemically reactive site.

Preferably the support is in the form of a bead. The bead may be of diameter 0.5μm to 1cm. The bead may be a gel type polymer. The bead may have macroporous structure. The bead polymer matrix structure may be highly crosslinked.

The synthetic chemistry may be performed using a single bead, or many beads.

The support may be a gel type polymer.

Preferably the combinatorial chemistry strategy includes at least one step wherein a radiolabelled receptor molecule is incubated with a support. Preferably, the receptor molecule is biologically active. Preferably the support is in the form of beads, and each bead bearing a potential ligand for a receptor molecule, with different beads bearing different ligands. After incubation, the biologically active molecule, having great affinity for the ligand(s) attached to the bead(s), will be bound to the bead(s) bearing the ligand(s). The binding of the radiolabelled receptor molecule to the bead(s) incorporating scintillant moieties will result in activation of these scintillant moieties. The beads bearing the most active ligands will thus display the most scintillational fluorescence. A main library and positionally fixed sub-libraries may be synthesised

and the scintillation emitted by each library and sub-library used to show: 1) that the main library contains one or more molecules that bind to the receptor molecule; and 2) identify the most active sublibraries and thus identify the most active library compound directly. For example, it is possible to synthesise a tetrapeptide on a scintillant resin. Subsequent binding of a radiolabelled biological molecule may then be monitoredto determine the extent and kinetics of binding of the molecule with the tetrapeptide . In a different strategy, it is possible to use a scintillation based assay to identify the most active bead, and deduce the identity of the compound on that bead by employing conventional encoding strategies.

This application of the supports of the present invention has the advantage that it can be the ligand which is on the support and this is added to a solution containing the radiolabelled biologically active molecule. This is in direct contrast to the use of conventional SPA beads, in which the biological receptor is linked to SPA beads and these SPA beads are added to a solution containing a potential ligand which is radiolabelled. By use of the present invention, a single radiolabelled receptor compound may be screened against as many potential ligands on the support as required. Radiolabelling is thus kept to a minimum and there is no requirement for a known ligand to be available in radiolabelled form.

The supports of the present invention have the flexibility to be used in conventional SPA style assays too, in which the receptor is linked to the support and the radiolabel is to be found in

the ligand in solution.

The scintillant supports of the present invention permit simultaneous assay and deconvolution for libraries of compounds synthesised on the supports. Sublibraries may be positionally fixed and the assay procedure may be by direct scintillation counting to detect binding interactions or by scintillation counting after a washing or dilution procedure.

In a further aspect, the present invention provides a method for determining how many chemically reactive sites there are on or within a scintillant solid support, incorporating the steps of:

[a] providing a known amount of support for a chemical reaction comprising a polymer matrix having at least one scintillant moiety and at least one chemically reactive site, the scintillant moiety being covalently bonded into the polymer matrix;

[b] mixing the support with a molecule comprising a site which may bind/react with the chemically reactive site on the support and an activating group; and

[c] measuring the scintillation produced by the scintillant moiety.

By activating group it is meant a group which will activate the scintillant moiety and cause it to scintillate.

The method can be used to determine the number of reactive sites per unit volume, per unit area or per unit mass.

A method of monitoring the progress of a chemical reaction comprises the steps of:

[a] providing an activating group and a known amount of support for a chemical reaction comprising a polymer matrix having at least one scintillant moiety and at least one chemically reactive site, the scintillant moiety being covalently bonded into the polymer matrix, the chemically reactive site being bound to a reactant molecule comprising a site which binds with the chemically reactive site on the support;

[b] measuring the scintillation produced by the scintillant moiety;

[c] subjecting the support to reaction conditions whereby the activating group is removed from the reactant molecule such that the activating group is removed from the support; and

[d] measuring the scintillation produced by the scintillant moiety.

By activating group it is meant a group which will activate the scintillant moiety and cause it to scintillate.

In a still a further embodiment of the invention, a method of monitoring the progress of a chemical reaction comprises the

steps of:

[a] providing a known amount of support for a chemical reaction comprising a polymer matrix having at least one scintillant moiety and at least one chemically reactive site, the scintillant moiety being covalently bonded into the polymer matrix;

[b] mixing the support with a molecule comprising a site which may bind/react with the chemically reactive site on the support and an activating group; and

[c] measuring the scintillation produced by the scintillant moiety.

Embodiments of the invention will now be described. It will be appreciated that embodiments of the invention can be used according to the methods described in the drawings, in which:

Figure 1A is a schematic representation of the 'split and mix' method;

Figure 1B is a schematic representation of positionally fixed library synthesis;

Figure 2 is a graph of scintillation counting results obtained for the scintillant gel type resin synthesized using monomer 5;

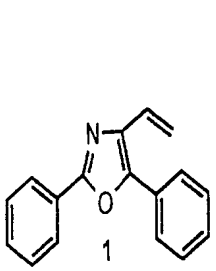
Figure 3 is a graph of scintillation counting results obtained

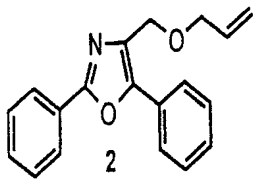
for the scintillant macroporous resin synthesized using monomer 5; and

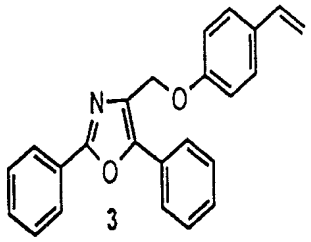
Figure 4 is a graph of scintillation counting results obtained for the aqueous compatible resin of Example 15.

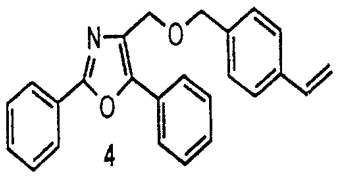
Examples 1-5: Synthesis of Scintillant Monomers

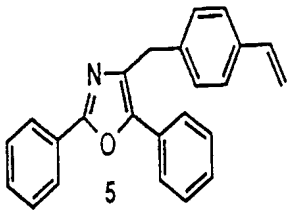
Monomers (1-5) contain the 2,5-diphenyloxazole moiety. 2,5-Diphenyloxazole is a well known scintillant and these monomers are termed 'scintillant monomers'. The synthetic route to each of these monomers is outlined in scheme 1.











The synthetic details required to synthesise the starting materials (aldehyde 6, alcohol 7 and bromide 8) are published at *Tetrahedron Lett.*, 1997, 38, 52, 9061.

Synthesis of Scintillant Monomers and Precursors

2,5-Diphenyl-4-hydroxymethyloxazole(alcohol 7)

Lithium borohydride (1M solution in THF , 280 ml, 280 mmol) was added, over a 0.5 h period, to a stirred solution of ethyl 2,5-diphenyloxazole-4-carboxylate (78.6 g, 0.27 mol) in THF (100 ml) at 0°C under an atmosphere of nitrogen. Lithium triethylborohydride (1M solution in THF, 28.0 ml, 28.0 mmol) was added and the mixture was stirred at room temperature for a further 3 h. Hydrochloric acid (2M) was added cautiously until no further effervescence was observed, and then an aqueous solution of sodium hydroxide (2M, 200 ml) was added to make the aqueous phase slightly basic. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (200 ml). The combined organic phases were washed with a saturated aqueous solution of ammonium chloride (200 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to furnish 2,5-diphenyl-4-hydroxymethyloxazole (66.1 g, 98%) as a yellow solid; M.Pt. 126-128°C; ν_{max} (cm^{-1}) 3 252 (br, m), 3 056 (w), 2 925 (w), 2 875 (w), 1 589 (w), 1 548 (w), 1 486 (m), 1 446 (w), 1 026 (m), 1 007 (m), 776 (m), 704 (s), 688 (s); δ_H (270 MHz, $CDCl_3$) 4.86 (2 H s), 7.37-7.51 (6 H m), 7.74 (2 H dd *J* 8.3, 1.6), 8.03-8.07 (2 H m); δ_C (67.5 MHz, $CDCl_3$) 57.1, 126.1, 126.4, 127.0, 128.1, 128.6, 128.8, 128.9, 130.5, 136.1, 147.3, 160.0; MS (APCI, *m/e*) 252.5 (M + 1).

4-Bromomethyl-2,5-diphenyloxazole (bromide 8)

Phosphorous tribromide (1.40 ml, 14.7 mmol) was added to a solution of 2,5-diphenyl-4-hydroxymethyloxazole (10.0 g, 39.8 mmol) in dichloromethane (200 ml). The resultant mixture was stirred at room temperature for 2 h before a small portion of brine was added to quench any excess phosphorous tribromide. The organic phase was then washed with brine (200 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to furnish 4-bromomethyl-2,5-diphenyloxazole (10.3 g, 82%) as a pale yellow solid; M.Pt. 160-161^{0}C, ν_{max} (cm^{-1}) 3 413 (w), 3 032 (w), 2 976 (w), 1 592 (w), 1 552 (w), 1 484 (m), 1 446 (m), 1 212 (m), 1 068 (w), 907 (w), 769 (m), 701 (s), 686 (s), 665 (s); δ_H (270 MHz, $CDCl_3$) 4.67 (2 H s), 7.39-7.56 (6 H m), 7.79 (2 H dd *J* 3.6, 1.7), 8.08-8.12 (2 H m); δ_C (67.5 MHz, $CDCl_3$) 25.5, 126.2, 126.5, 126.9, 127.7, 128.8, 129.0, 129.1, 130.7, 133.6, 147.7, 160.1; MS (APCI, *m/e*) 314.6 (M + 1), 316.7 (M + 1).

4-Carboxaldehyde-2,5-diphenyloxazole(aldehyde 6)

2-Iodoxybenzoic acid (2.67 g, 9.27 mmol) was added to a solution of 4-hydroxymethyl-2,5-diphenyloxazole (2.00 g, 7.97 mmol) in DMSO (40 ml). The resultant mixture was stirred for 3 h before water (160 ml) was added. The product was extracted into diethyl ether (3x 50 ml). The combined organic extracts were washed with an aqueous solution of sodium hydroxide (2M, 2x 100 ml), water (5x 50 ml), brine (2x 50 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to furnish 4-carboxaldehyde-2,5-diphenyloxazole (1.84 g, 93%); M.Pt. 102-103^{0}C, ν_{max} (cm^{-1}) 3 060

(w), 2 837 (w), 2 766 (w), 1 695 (s), 1 563 (m), 1 548 (m), 1 490 (m), 1 448 (m), 1 069 (w), 1 025 (w), 777 (s), 710 (s), 688 (s); δ_H (270 MHz, $CDCl_3$) 7.51-7.55 (6 H m), 8.12-8.16 (4 H m), 10.16 (1 H s); δ_C(67.5 MHz, $CDCl_3$) 126.0, 126.2, 126.8, 127.7, 128.9, 129.0, 131.2, 131.3, 135.5, 155.9, 160.3, 185.1; MS (APCI, *m/e*) 250.5 (M + 1).

2,5-Diphenyl-4-vinyloxazole (monomer 1)

A solution of methyltriphenylphosphonium bromide (3.17 g, 8.87 mmol) and sodium methoxide (0.48 g, 8.89 mmol) in THF (60 ml) and DMF (10 ml) was cooled to 0^{0}C and stirred for 4 h under an atmosphere of nitrogen. The solution was allowed to warm to room temperature and a solution of 4-carboxaldehyde-2,5-diphenyloxazole (1.84 g, 7.39 mmol) in THF (30 ml) was added. The resultant solution was then stirred for 14 h at room temperature before being poured into brine (50 ml). The organic layer was separated and the aqueous phase extracted with diethyl ether (2x 50 ml). The combined organic extracts were then washed with brine (5x 50 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to furnish the crude product as a brown solid (4.72 g). Purification by column chromatography (gradient elution; 0-3% ethyl acetate/light petroleum) gave 2,5-diphenyl-4-vinyloxazole (1.37 g, 74%) as a white solid; M.Pt. 70-71^{0}C, ν_{max} (cm^{-1}) 3 051 (w), 1 830 (w), 1 636 (w), 1 598 (m), 1 558 (m), 1 488 (s), 1 444 (s), 1 411 (w), 1 357 (w), 1 245 (w), 1 084 (m), 1 070 (m), 1 027 (m), 979 (m), 945 (m), 912 (m), 778 (m), 703 (m), 648 (m); δ_H (270 MHz, $CDCl_3$) 5.47 (1 h dd *J* 10.9, 2.0), 6.28 (1 H dd *J* 17.2, 2.0), 6.97 (1 H dd *J* 16.8, 10.9), 7.30-7.48 (6 H m), 7.64-7.69 (2 H m), 8.09-8.14 (2 H m);δ_C

(67.5 MHz, $CDCl_3$) 117.5, 125.8, 126.4, 126.6, 127.2, 128.4, 128.6, 128.7, 128.8, 130.4, 135.2, 146.1, 160.0; MS (APCI, *m/e*) 248.2 (M + 1).

Allyl-2,5-diphenyloxazole-4-methylether (monomer 2)

Sodium hydride (55% dispersion in oil; 0.35 g, 8.02 mmol) was washed with light petroleum (2x 5 ml) under a nitrogen atmosphere. A solution of 2,5-diphenyl-4-hydroxymethyloxazole (1.00 g, 3.98 mmol) in THF (40 ml) was added to the sodium hydride and the mixture was stirred at room temperature for 2 h. Allyl bromide (0.56 ml, 6.03 mmol) was then added and the resulting solution was stirred at room temperature for a further 14 h. An aqueous solution of saturated ammonium chloride (30 ml) was added cautiously, the organic layer was separated and the aqueous phase extracted with diethyl ether (30 ml). The combined organic extracts were washed with brine (30 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to furnish a yellow solid (1.89 g). Purification by column chromatography (gradient elution; 0-20% ethyl acetate/light petroleum) gave allyl-2,5-diphenyloxazole-4-methylether (0.91 g, 78% yield) as a white solid; M.Pt. 71-72°C, ν_{max} (cm^{-1}) 3 432 (w), 3 064 (w), 3 014 (w), 2 919 (w), 2 840 (w), 1 646 (w), 1 590 (w), 1 547 (m), 1 494 (m), 1 448 (m), 1 280 (w), 1 146 (w), 1 084 (s), 1 057 (s), 912 (s), 778 (s), 759 (s), 708 (s), 688 (s); δ_H (270 MHz, $CDCl_3$) 4.16-4.19 (2 H m), 4.66 (2 H s), 5.25 (1 H dd *J* 10.2, 1.6), 5.38 (1 H dd *J* 17.5, 1.6), 5.94-6.08 (1 H m), 7.35-7.51 (6 H m), 7.80 (2 H dd *J* 8.2, 1.3), 8.10-8.15 (2 H m); δ_C (67.5 MHz, $CDCl_3$) 64.4, 71.5, 117.8, 126.3, 126.4, 127.3, 128.2, 128.6, 128.8, 128.9, 130.4, 134.0, 134.4, 148.9, 159.8; MS

(APCI, *m/e*) 291.9 (M + 1), 292.9 (M + 2).

2,5-diphenyl-4-methyloxazole(4-formylphenyl)ether (precursor to monomer 3)

A mixture of 4-bromomethyl-2,5-diphenyloxazole (4.30 g, 13.7 mmol), 4-hydroxybenzaldehyde (1.67 g, 13.7 mmol) and potassium carbonate (2.08 g, 15.1 mmol) were refluxed in butanone (200 ml) for 4 h. After cooling to room temperature, the mixture was filtered through a pad of celite and concentrated under reduced pressure to furnish a dark brown solid (4.40 g). Purification by column chromatography (gradient elution; 5-20% ethyl acetate/light petroleum) gave 2,5-diphenyl-4-methyloxazole(4-formylphenyl) ether (1.16 g, 24%) as a white solid; M.Pt. 109-110^{0}C; ν_{max} (cm^{-1}) 3 057 (w), 2 840 (w), 2 752 (w), 1 692 (s), 1 604 (s), 1 578 (s), 1 551 (m), 1 486 (m), 1 446 (m), 1 391 (m), 1 313 (m), 1 247 (s), 1 164 (s), 1 091 (w), 1 068 (w), 997 (s), 868 (s), 839 (m), 744 (m), 702 (s), 688 (s); δ_H (270 MHz, $CDCl_3$) 5.27 (2 H s), 7.21 (2 H d *J* 8.9), 7.41-7.51 (6 H m), 7.73-7.77 (2 H m), 7.87 (2 H d *J* 8.6), 8.10-8.14 (2 H m), 9.90 (1 H s); δ_C (67.5 MHz, $CDCl_3$) 63.1, 115.3, 126.3, 126.5, 127.0, 127.7, 128.9, 129.1, 130.3, 130.7, 131.7, 132.0, 149.8, 160.1, 163.4, 190.8; MS (APCI, *m/e*) 356.3 (M + 1).

2,5-Diphenyl-4-methyloxazole(4-vinylphenyl) ether (monomer 3)

A solution of methyltriphenylphosphonium bromide (4.28 g, 12.0 mmol) and sodium methoxide (0.65 g, 12.0 mmol) in THF (30 ml) and DMF (20 ml) was cooled to 0^{0}C and stirred for 4 h under an atmosphere of nitrogen. The solution was allowed to warm to room temperature and a solution of 2,5-diphenyl-4-

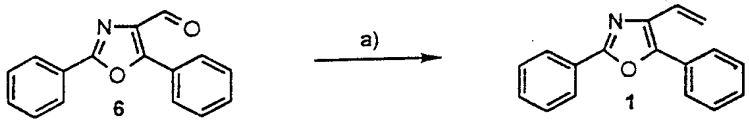
methyloxazole(4-formylphenyl) ether (3.55 g, 10.0 mmol) in THF (30 ml) was added. The resultant solution was stirred at room temperature for 14 h before being poured into brine (50 ml). The organic layer was separated and the aqueous phase extracted with diethyl ether (2x 50 ml). The combined organic extracts were washed with brine (5x 50 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to furnish a brown solid (6.15 g). Purification by column chromatography (gradient elution; 5-15% ethyl acetate/light petroleum) gave 2,5-diphenyl-4-methyloxazole(4-vinylphenyl) ether (1.57 g, 44%) as a white solid; M.Pt. 112-113°C; ν_{max} (cm^{-1}) 3 433 (w), 3 056 (w), 2 926 (w), 2 856 (w), 1 546 (m), 1 498 (m), 1 456 (m), 1 378 (w), 1 279 (w), 1 076 (s), 987 (m), 905 (m), 824 (m), 780 (m), 759 (m), 706 (s), 688 (s); δ_H (270 MHz, $CDCl_3$) 5.17 (1 H dd *J* 10.9, 1.0), 5.19 (2 H s), 5.67 (1 H dd *J* 17.8, 1.0), 6.73 (1 H dd *J* 17.5, 10.9), 7.06 (2 H dd *J* 6.9, 2.3), 7.35-7.51 (8 H m), 7.78 (2 H dd *J* 6.6, 1.6), 8.09-8.16 (2 H m); δ_C (67.5 MHz, $CDCl_3$) 62.9, 111.9, 115.0, 126.3, 126.5, 127.2, 127.4, 127.9, 128.8, 128.9, 129.0, 130.5, 131.0, 132.5, 136.2, 149.6, 158.2; MS (APCI, *m/e*) 354.1 (M + 1).

2,5-Diphenyl-4-methyloxazole (4-vinylbenzyl) ether (monomer 4)

Sodium hydride (55% dispersion in oil; 1.00 g, 22.9 mmol) was washed with light petroleum (2x 5 ml) under a nitrogen atmosphere. A solution of 4-hydroxymethyl-2,5-diphenyloxazole (2.00 g, 7.97 mmol) in THF (50 ml) was added to the sodium hydride and the mixture was stirred at room temperature for 2 h. 4-Vinylbenzylchloride (1.35 ml, 9.58 mmol) was then added

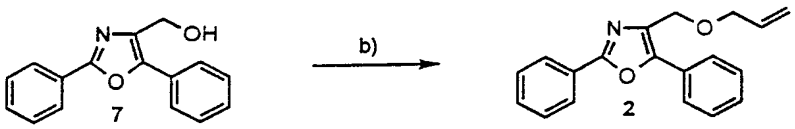
and the resulting solution was stirred at room temperature for 14 h. DMF (30 ml) was added and stirring continued for a further 24 h at room temperature. An aqueous solution of saturated ammonium chloride (30 ml) was added cautiously, the organic layer was separated and the aqueous phase extracted with diethyl ether (2x 50 ml). The combined organic extracts were washed with brine (5x 50 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to furnish a yellow solid (4.00 g). Purification by column chromatography (gradient elution; 0-10% ethyl acetate/light petroleum) gave 2,5-diphenyl-4-methyloxazole (4-vinylbenzyl) ether as a white solid (2.21 g, 76%); M.Pt. 97-98°C; ; ν_{max} (cm^{-1}) 3 432 (w), 2 926 (w), 1 605 (m), 1 509 (s), 1 240 (s), 1 175 (m), 1 003 (m), 897 (m), 834 (m), 709 (m), 685 (m), 668 (m); δ_H (270 MHz, $CDCl_3$) 4.68 (4 H s), 5.27 (1 H dd *J* 11.9, 1.0), 5.79 (1 H dd *J* 11.5, 1.0), 6.77 (1 H dd *J* 11.5, 10.9), 7.34-7.50 (6 H m), 7.71-7.74 (2 H m), 8.08-8.16 (2 H m); δ_C (67.5 MHz, $CDCl_3$) 64.2, 113.8, 113.9, 126.1, 126.2, 126.4, 127.3, 128.1, 128.5, 128.7, 128.8, 130.4, 133.9, 136.5, 137.1, 137.4, 148.9, 159.7; MS (APCI, *m/e*) 368 (M + 1).

Monomer 1



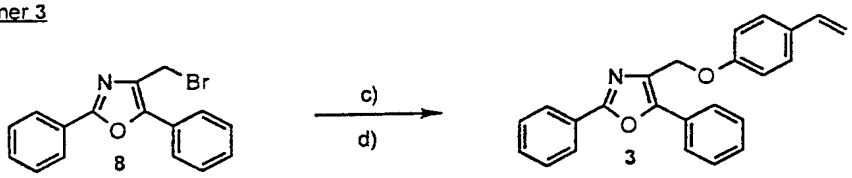
Reaction conditions: a) $Ph_3P^+CH_3\ Br^-$, NaOMe, THF, DMF

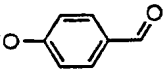
Monomer 2



Reaction conditions: b)  , NaH, THF

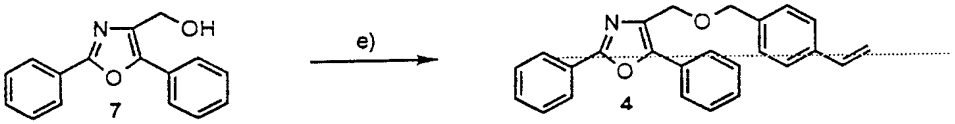
Monomer 3

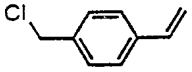


Reaction conditions: c)  , K_2CO_3, Butanone, Δ;

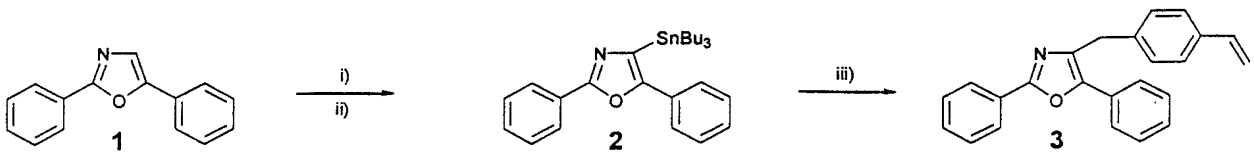
d) $Ph_3P^+CH_3\ Br^-$, NaOMe, THF, DMF

Monomer 4



Reaction conditions: e)  , NaH, THF

Example 5:Synthesis Route to Monomer 5



Reagents: i) 2,2,6,6-tetramethylpiperidine, *sec*-butyllithium, THF; ii) $[CH_3(CH_2)_3]_3SnCl$, THF; iii) $(C_6H_5CH=CHCOCH=CHC_6H_5)_3Pd_2$, $(C_6H_5)_3As$, CuO, 1-methyl-2-pyrrolidinone, 4-vinylbenzyl chloride.

Scheme 1a

2,5-Diphenyl-4-tributyltinoxazole 2

To a solution of 2,5-diphenyloxazole 1 (8.84g, 40.0 mmol) and tetramethylpiperidine (0.68 cm^3; 0.57 g, 4.0 mmol) in tetrahydrofuran (150 cm^3) at -78°C, was added *sec*-butyllithium (1.3M solution in cyclohexane, 33.8 cm^3, 44.0 mmol) dropwise over 30 min. The solution of 4-lithio-2,5-diphenyloxazole that resulted was allowed to warm to 0°C before being transferred, via cannula, into a solution of tributyltin chloride (10.9 cm^3; 13.1 g, 40.0 mmol) in tetrahydrofuran (100 cm^3) at -78°C. The reaction mixture was stirred at -78°C for fifteen minutes before being allowed to warm to room temperature. After stirring for a further 30 minutes, the reaction was quenched by the addition of a saturated, aqueous solution of ammonium chloride (100 cm^3). The organic layer was separated, dried over magnesium sulfate and concentrated under reduced pressure. Purification by flash column chromatography (gradient elution 0-3% ether in hexane) furnished 2,5-diphenyl-4-tributyltinoxazole 2 (15.4 g, 75%) as a colourless oil; ν_{max}(thin film, NaCl plates)/ cm^{-1} 2955, 2924, 2851, 1542, 1481,

1447, 1376, 1339, 1068, 1024, 972, 875, 774, 762, 709 and 690; d_H (300 MHz; $CDCl_3$) 0.85-1.60 (27H, m), 7.34-7.48 (6H, m), 7.66-7.69 (2H, m) and 8.14 (2H, dd, *J* 8.3, 1.8); d_C (75 MHz; $CDCl_3$) 10.4, 13.7, 27.2, 29.0, 125.8, 126.4, 128.1, 128.6, 128.65, 129.7, 130.2, 132.0, 136.4, 157.8, 161.7; MS (APCI, m/e) 512 (M+1).

(4'-Vinyl)-4-benzyl-2,5-diphenyloxazole 3 (Monomer 5)

A solution of tris-(dibenzylidineacetone)dipalladium(0) (23.0 mg, 25.0 mmol, 5 mol% Pd), triphenylarsine (61 mg, 200mmol, 20 mol% ligand) and copper oxide (79.0 mg, 1.00 mmol) in 1-methyl-2-pyrrolidinone (10 cm^3) were stirred together at room temperature for 15 minutes. To this stirred solution, 4-vinylbenzylchloride (157 ml; 170 mg, 1.11 mmol) was added and the resultant mixture stirred for a further 15 minutes before a solution of 2,5-diphenyl-4-tributyltinoxazole (612 mg, 1.20 mmol) in 1-methyl-2-pyrrolidinone (10 cm^3) was added. The resultant mixture was warmed to 65 °C and stirred for 4 hours after which time blackening of the mixture had occurred. After cooling to room temperature, diethyl ether (25 cm^3) and a 10% aqueous solution of potassium fluoride (75 cm^3) were added and the mixture was stirred for one hour. The mixture was then filtered through a pad of Celite® and the pad was then washed with diethyl ether (25 cm^3). The aqueous phase was separated and extracted with a further portion of diethyl ether (25 cm^3). The combined organic extracts were washed with a saturated aqueous solution of ammonium chloride (4 x 25 cm^3), dried over magnesium sulfate and concentrated under reduced pressure to yield a yellow solid. Purification by flash column chromatography (eluent: 10% ether in hexane) furnished (4'-

vinyl)-4-benzyl-2,5-diphenyloxazole 3 as a white solid (321 mg, 86%). M.Pt. 106-108 °C; ν_{max}(KBr)/ cm^{-1} 3059, 2925, 1486, 1448, 1068, 955, 901, 820, 774, 708 and 689; d_H (300 MHz; $CDCl_3$) 4.28 (2 H, s), 5.27 (1 H, d, *J* 10.9), 5.77 (1 H, d, *J* 17.6), 6.78 (1 H, dd, *J* 17.6, 10.9), 7.35-7.54 (10 H, m), 7.74-7.77 (2 H, m) and 8.20-8.22 (2 H, m); d_C (75 MHz; $CDCl_3$) 32.8, 113.1, 125.4, 125.8, 126.2, 126.3, 127.3, 127.8, 128.4, 128.5, 128.7, 130.0, 135.5, 135.7, 136.4, 138.1, 146.3 and 159.7; MS (APCI, m/e) 338 (M+1).

Chemically inert scintillant solid supports may be constructed by co-polymerising a scintillant monomer (1-5) with a chemically un-functionalised monomer or a chemically functionalised monomer. The covalent incorporation of scintillant molecules into the polymer matrices of these solid supports enables their use in all solvents, without leaching of the scintillant molecules. Consequently, these supports will retain the ability to scintillate strongly in the presence of ionising radiation (for example β particles and auger electrons), even if they have been used previously to carry out solid phase synthetic chemistry.

Example 6: Synthesis of Scintillant Merrifield's Resin

By Merrifield's resin it is meant a chloromethyl polystyrene resin which may be macroporous or gel type resin. Merrifield's resin is a commercially available polystyrene-based, beaded form of solid support, used widely in solid phase synthetic chemistry. Chemically reactive benzyl chloride groups are distributed randomly throughout each resin bead. When the

beads are used for solid phase synthesis, each solvent-accessible benzyl chloride group reacts in identical fashion. Merrifield's resin is constructed in a free radical initiated, suspension co-polymerisation reaction of styrene, chloromethylvinyl benzene and divinylbenzene.

An analogous suspension polymerisation reaction which additionally contains one of the scintillant monomers (1-5) yields a Merrifield's resin which also contains scintillant molecules incorporated covalently into the polymer matrix of each resin bead. This can be termed 'scintillant Merrifield's resin'. Even after scintillant Merrifield's resin beads have been used for solid phase synthetic chemistry, the covalent incorporation of scintillant ensures that they retain the ability to scintillate in the presence of ionising radiation even after prolonged exposure to organic solvents. As a support for carrying out solid phase synthetic chemistry, Merrifield's resin and scintillant Merrifield's resin are essentially interchangeable.

Scintillant monomer (1) is co-polymerised with 4-ethylvinylbenzene, divinylbenzene and 4-vinylbenzyl chloride. AIBN (2,2' azobisisobutyronitrile) was used as a free radical initiator to start the reaction and toluene was used as a porogen. A standard suspension polymerisation procedure was carried out to produce a highly cross-linked Merrifield's resin. Unreacted monomers and any impurities were removed from the product polymer by exhaustive soxhlet extraction.

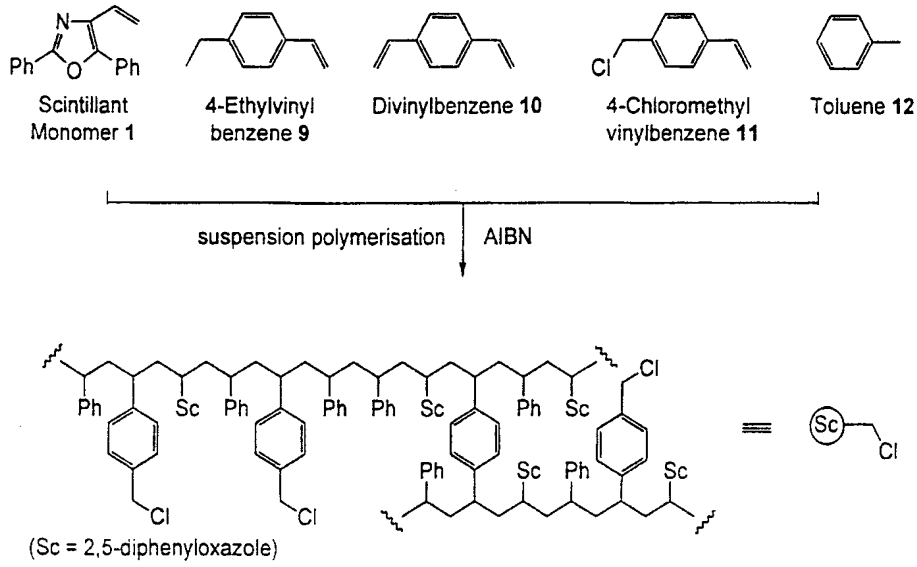
The reaction scheme is shown in Scheme 2 and full experimental

details of the reaction are given below.

Scintillant monomer (1)	= 2.60 g, 10.5 mmol
4-Ethylvinylbenzene (9) (co-monomer)	= 4.91 ml, 4.49 g, 34.0 mmol
Divinylbenzene (10) (used as a cross-linking agent)	= 6.00 ml, 5.48 g, 42.1 mmol
4-Vinylbenzyl chloride (11) (functionalised monomer)	= 2.62 ml, 2.84 g, 18.6 mmol
Toluene (12) (used as a porogen)	= 13.53 ml (equal to the combined monomer volume.)
AIBN (used as a radical initiator)	= 0.250 g

Each of the reagents listed above were added to a bulk aqueous phase (250 ml) that contained 87-89% hydrolysed polyvinylalcohol (2.5 g) (acts as a droplet stabiliser).

Synthesis of Scintillant Merrifield's Resin



Scheme 2

ST

The mixture was placed under a nitrogen atmosphere, stirred at 500 rpm and then heated to 80°C to initiate thermal decomposition of the radical initiator and thus start the polymerisation reaction. Stirring and heating were maintained for a further twelve hours, after which time all of the organic droplets in the bulk aqueous phase had solidified. The mixture was cooled to room temperature, filtered and washed (water 3x 200 ml followed by ethanol 3x 200 ml) to furnish 3.91 g of a beaded product of between 300-500 μm in diameter. The beads were then soxhlet extracted for two successive periods of 8 hours with tetrahydrofuran (250 ml).

Example 7: Evaluation of Scintillant Merrifield's Resin by Scintillation Counting

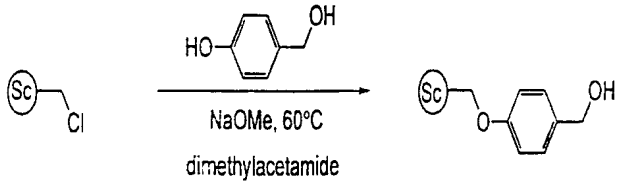
Portions of scintillant Merrifield's resin obtained in Example in 6 were placed in 0.5 ml Eppendorf tubes. To each portion of beads, an aliquot of a stock solution of ^{14}C labelled hexadecane in toluene was added. Each tube was then monitored in a scintillation counter. As a control experiment, the total amount of radioactivity (maximum counts per minute (CPM)) per aliquot was determined by counting in Ultima Gold (RTM), a commercial scintillation cocktail. The results obtained are tabulated below and indicate that even after prolonged periods of soxhlet extraction the scintillant molecules remain within the resin beads. This finding indicates that the scintillant molecules are covalently incorporated into the polymer matrix, and that the support may subsequently be used in organic solvents without leaching of the scintillant molecules occurring.

EXPT.	Mass of scintillant Merrifield's resin used /mg	TOTAL CPM	CPM /mg resin
Soxhlet time = 0hrs	5.05	36 472	7 222
	7.18	43 044	5 995
	7.31	46 582	6 372
			average = 6 530
Soxhlet time = 8hrs	7.19	35 462	4 932
	6.64	33 052	4 977
	7.65	37 433	4 893
			average = 4 934
Soxhlet time = 16hrs	5.82	35 535	6 016
	7.32	40 923	5 591
	7.06	40 418	5 725
			average = 5 807
Ultima Gold		122 632	
(represents maximum		120 887	
possible counts)		121 432	
		average = 121 650	

Example 8: Synthesis of Scintillant Wang Resin

Scintillant Merrifield's resin obtained in Example 6 has been derivatised into scintillant Wang resin. The following experimental procedure was employed, and is outlined in scheme 3:

Synthesis of Scintillant Wang Resin



Scheme 3

Hydroxybenzyl alcohol (0.45 g, 3.62 mmol) and sodium methoxide (0.19 g, 3.52 mmol) were placed under an atmosphere of nitrogen and dissolved in dimethylacetamide (25 ml). After stirring this solution for 30 minutes it was transferred into a second flask containing scintillant Merrifield's resin (300-500μm in diameter) of Example 6, (1.00 g, approx. 1.2 mmol). The resulting mixture was stirred at 60°C for 14 hrs. After cooling to room temperature, the beads were collected by filtration and washed with dioxane (100 ml), dioxane:water (3:1, 100 ml), dioxane (100 ml) and methanol (100 ml). The beads were then dried under vacuum to yield 1.07 g of scintillant Wang resin.

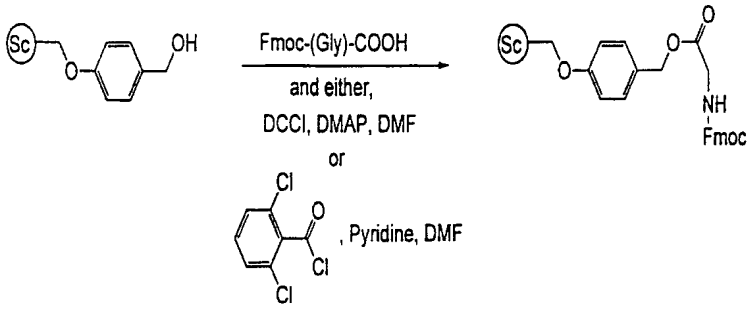
In a non-quantified experiment, an aliquot of ^{14}C labelled

hexadecane in toluene was added to a portion of the scintillant Wang resin and the mixture counted in a scintillation counter. The resin scintillated strongly, indicating that the scintillant molecules remain covalently incorporated into the polymer matrix of the resin.

Example 9 : Reaction of Scintillant Wang Resin of Example 8 with Fmoc-(Gly)-COOH

The reaction pathway is shown in scheme 4a.

Reaction with Fmoc-(Gly)-COOH



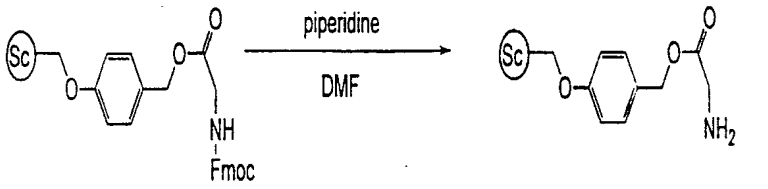
Scheme 4a

Scintillant Wang resin of Example 8 has been coupled to an Fmoc protected amino acid. The success of this reaction demonstrates that scintillant resins can be coupled to amino acids, and indicates that it is possible to use scintillant resins to carry out solid phase synthesis. The full experimental details are given below.

Scintillant Wang resin (0.20 g, approx. 0.2 mmol) and Fmoc-Glycine (0.178 g, 0.6 mmol) were placed under an atmosphere of nitrogen and stirred in DMF (5 ml). After 30 minutes, 2,6-dichlorobenzoyl chloride (0.085 ml, 0.6 mmol) and pyridine (0.100 ml, 1.24 mmol) were added to the mixture. Stirring was continued for a further 14 hours after which time the beads were collected by filtration. The resin beads were washed *in situ* with dichloromethane (30 ml) followed by methanol (30 ml). The beads were then dried under vacuum to yield 0.210 g of the derivatised resin.

The beads were then subjected to standard Fmoc cleavage conditions (scheme 4b)(piperidine in DMF, NOVA BioChem 97/98 Catalogue page S37) and the average loading of the Fmoc-Glycine on the resin calculated. The resin loading was 0.15 mmol / gram of resin beads.

Cleavage of Fmoc group

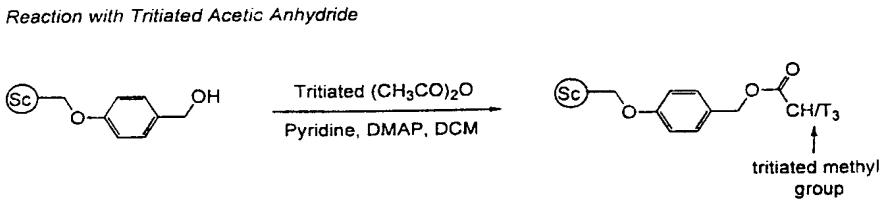


Cleaved Fmoc group detected and quantified by UV/vis spectroscopy

Scheme 4b

Example 10: Reaction of Scintillant Wang Resin of Example 8 with Tritiated Acetic Anhydride

The reaction pathway is shown in scheme 5.



Scheme 5

Scintillant Wang resin of Example 8 (0.123 g), dimethylaminopyridine (DMAP) (catalytic amount) and pyridine (1 ml) were stirred in dichloromethane (5 ml). To this mixture, tritiated acetic anhydride (0.100 ml, 1.06 mmol, 10 μCi) was added. The reaction mixture was stirred for six hours at room temperature. After this time, the resin beads were collected by filtration. The beads were washed in situ, extensively with successive portions of dichloromethane (3x 10 ml), dichloromethane:methanol (50:50, 3x 10 ml), methanol (3x 10 ml), dichloromethane:methanol (50:50, 3x 10 ml) and finally dichloromethane (3x 10 ml). The beads were then left to dry in situ for 12 hours. Approximately 7 mg portions of the beads were placed in scintillation vials before being counted in the scintillation counter. The following counts per minute (CPM) per mg of resin were measured as shown in column 4 of the following table.

When Ultima Gold (10 ml), a commercial scintillation

cocktail, was added to each tube, the CPM per mg of resin increased (see column 5 of the table).

Tube	Mass of tritiated resin/mg	cpm	cpm/mg of resin	cpm/mg of resin in Ultima Gold
1	6.39	3 396	532	1072
2	8.17	4 699	575	1086
3	7.31	4 287	586	995
4	7.78	4 300	552	841
5	7.33	3 901	532	867

This gave an average cpm/mg of tritiated resin of 555 cpm/mg and an average cpm/mg in Ultima Gold of 972. The beads are thus 57% as efficient at counting as the commercial scintillation cocktail Ultima Gold. When these figures are used to work out the degree of loading on the precursor scintillant Wang resin a loading of 0.137 mmol/g of resin is obtained. This value is in excellent agreement with the value of 0.15 mmol/g obtained by standard methods, described in Example 9.

It is thus demonstrated that covalent attachment of a radiolabel to a scintillant resin according to the present invention causes scintillation, with an efficiency of scintillation counting of 57% relative to a commercial scintillation cocktail. The scintillation based method to detect and quantify binding interactions by scintillation counting is thus viable.

Example 12:Synthesis of Scintillant Resins with Monomer 5

Synthesis of Gel-type Scintillant Resins using Monomer 5

Gel-type 1% cross linked chloromethyl polystyrene resin, containing 2.0% oxazole

A suspension polymerisation reactor vessel fitted with a PTFE propeller was filled with a 1% solution of polyvinylalcohol (87-89% hydrolysed, *Mn.* 100 000) (500 cm^3). The reactor was purged with nitrogen for 10 min before a solution consisting of styrene (63.0 cm^3 , 57.2 g, 549 mmol), 80 % divinylbenzene (2.30 cm^3 , 2.10 g, 12.9 mmol, {ethylstyrene, 3.23 mmol}), 4-vinylbenzylchloride (9.94 cm^3 , 10.76 g, 70.5 mmol), azobisisobutyronitrile (0.70g, 4.27 mmol) and (4'-vinyl)-4-benzyl-2,5-diphenyloxazole (monomer 5) (4.37 g, 13.0 mmol) was added. The stirrer blade was positioned 15mm below the interface of the two liquids and stirring at 600 RPM was initiated. After stirring for 0.5 h the temperature of the vessel was raised to 70 °C and stirring was continued overnight. The collected cloudy polymer water mixture was allowed to cool and then poured over a 38 m stainless steel sieve, the polymeric material that remained on the sieve was carefully washed *in situ* with copious amounts of water until the effluent from the sieve became clear. The polymeric material was then transferred to a large sintered funnel and washed with water (2 x 500 cm^3), methanol (500 cm^3), tetrahydrofuran (2 x 500 cm^3), methanol:tetrahydrofuran 1:1 (500 cm^3) and methanol (500 cm^3). The polymer was dried *in situ* under suction for 0.5 h before being transferred to a soxhlet thimble.

Subsequent soxhlet extraction of the polymeric material was carried out for sixteen hours using dioxane as the eluting solvent. After soxhlet extraction, the polymeric material was transferred to a sintered funnel where it was washed with a solution of methanol:tetrahydrofuran 1:1 (500 cm^3) followed by methanol (2 x 500 cm^3). After drying *in situ* using suction for 0.5 h the polymeric material was transferred to a 500 cm^3 round bottomed flask and thoroughly dried on a rotary evaporator at 80 °C under reduced pressure for 4 h. Finally, the dried polymeric material was transferred to a sieve shaker and shaken for 4 h to give the scintillant gel-type resin beads in the size ranges: [> 250 μm, 2.01 g], 250 - 150 μm, 5.38 g], [150 - 75 μm, 24.33 g] and [75 - 38 μm, <1.0 g]; ν_{max}(KBr)/ cm^{-1} 3024, 2920, 1599, 1490, 1447, 1365, 1265, 1176, 1119, 1067, 1020, 906, 834, 756, 694 and 533.

Similarly, two other gel-type scintillant resins, containing 0.5% and 1% oxazole respectively, were constructed using the same procedure.

Gel type 1% cross link chloromethyl polystrene resin, containing 0.5% oxazole

Styrene (64.0 cm^3, 58.2 g, 559 mmol), 80 % divinylbenzene (2.30 cm^3, 2.10 g, 12.9 mmol, {ethylstyrene, 3.23 mmol}), 4-vinylbenzylchloride (9.94 cm^3 , 10.76 g, 70.5 mmol), azobisisobutyronitrile (0.70g, 4.27 mmol) and (4'-vinyl)-4-benzyl-2,5-diphenyloxazole (monomer 5) (1.09 g, 3.24 mmol) gave the scintillant gel-type resin beads in the size ranges: [> 250 μm, <1.0g], 250 - 150 μm, 3.20g], [150 - 75 μmm, 22.83 g] and [75 - 38 μm, <1.0 g]; ν_{max}(KBr)/ cm^{-1} 3024, 2919, 1600, 1491, 1448, 1368, 1264, 1182, 1068,

1025, 905, 835, 755, 697 and 535.

Gel type 1% cross link chloromethyl polystrene resin, containing 1.0% oxazole

Styrene (63.7 cm^3, 57.9 g, 556 mmol), 80 % divinylbenzene (2.30 cm^3, 2.10 g, 12.9 mmol, {ethylstyrene, 3.23 mmol}), 4-vinylbenzylchloride (9.94 cm^3, 10.76 g, 70.5 mmol), azobisisobutyronitrile (0.70g, 4.27 mmol) and (4'-vinyl)-4-benzyl-2,5-diphenyloxazole (monomer 5) (2.19 g, 6.49 mmol) gave scintillant gel-type resin beads in the size ranges: [> 250 μm, 1.19 g], 250 - 150 μm, 2.38 g], [150 - 75 μm, 24.80 g] and [75 - 38 μm, <1.0 g]; V_{max}(KBr)/cm^{-1} 3024, 2921, 1600, 1491, 1449, 1368, 1264, 1181, 1116, 1067, 1025, 905, 835, 755, 697 and 535.

Synthesis of Macroporous Scintillant Resins using Monomer 5

The same suspension polymerisation procedure that was used to construct the scintillant gel-type resins was employed in the construction of the following scintillant macroporous resins.

Macroporous, chloromethyl polystyrene resin containing 1.0% oxazole

Styrene (12.5 cm^3, 11.3 g, 109 mmol), 80 % divinylbenzene (20.3 cm^3, 18.6 g, 114 mmol, {ethylstyrene, 28.5 mmol}), 4-vinylbenzylchloride (4.40 cm^3, 4.76 g, 31.2 mmol), azobisisobutyronitrile (0.35g, 2.14 mmol), (4'-vinyl)-4-benzyl-2,5-diphenyloxazole (monomer 5) (0.96 g, 2.85 mmol) and toluene (37 cm^3) gave macroporous scintillant resin beads in the size ranges: [> 250 μm, 1.2 g], 250 - 150 μm,

3.87 g], [150 - 75 μm, 20.83 g] and [75 - 38 μm, <1.0 g]; V_{max}(KBr)/cm^{-1} 3023, 2920, 1600, 1490, 1446, 1363, 1265, 1118, 988, 901, 829, 794, 758, 697 and 538.

Macroporous, chloromethyl polystyrene, 2.0 % oxazole

Styrene (12.2 cm^3, 11.0 g, 106 mmol), 80 % divinylbenzene (20.3 cm^3, 18.6 g, 114 mmol, {ethylstyrene, 28.5 mmol}), 4-vinylbenzylchloride (4.40 cm^3, 4.76 g, 31.2 mmol), azobisisobutyronitrile (0.35g, 2.14 mmol), (4'-vinyl)-4-benzyl-2,5-diphenyloxazole (monomer 5) (1.92 g, 5.71 mmol) and toluene (37 cm^3) gave macroporous scintillant resin beads in the size ranges: [> 250 μm, 1.45 g], 250 - 150 μm, 4.79 g], [150 - 75 μm, 17.7 g] and [75 - 38 μm, <1.0 g]; V_{max}(KBr)/cm^{-1} 3020, 2916, 1597, 1488, 1446, 1359, 1265, 1182, 1119, 1020, 990, 901, 829, 793, 759, 694 and 538.

Macroporous, chloromethyl polystyrene, 4.0% oxazole

Styrene (11.5 cm^3, 10.5 g, 104 mmol), 80 % divinylbenzene (20.3 cm^3, 18.6 g, 114 mmol, {ethylstyrene, 28.5 mmol}), 4-vinylbenzylchloride (4.40 cm^3, 4.76 g, 31.2 mmol), azobisisobutyronitrile (0.35g, 2.14 mmol), (4'-vinyl)-4-benzyl-2,5-diphenyloxazole (monomer 5) (3.85 g, 11.4 mmol) and toluene (37 cm^3) gave macroporous scintillant resin beads in the size ranges: [> 250 μm, 1.97 g], 250 - 150 μm, 1.89 g], [150 - 75 μm, 23.05 g] and [75 - 38 mm, <1.0 g]; V_{max}(KBr)/ cm^{-1} 3020, 2916, 1597, 1488, 1358, 1259, 1176, 1119, 1020, 990, 901, 829, 793, 756, 694 and 533.

Macroporous, chloromethyl polystyrene, 8.0% oxazole

Styrene (10.2 cm^3, 9.26 g, 88.9 mmol), 80 % divinylbenzene (20.3 cm^3, 18.6 g, 114 mmol, {ethylstyrene, 28.5 mmol}), 4-vinylbenzylchloride (4.40 cm^3, 4.76 g, 31.2 mmol), azobisisobutyronitrile (0.35g, 2.14 mmol), (4'-vinyl)-4-benzyl-2,5-diphenyloxazole (momomer 5) (7.69g, 22.8 mmol) and toluene (37 cm^3) gave macroporous scintillant resin beads in the size ranges: [> 250 mm, <1.0 g], 250 - 150 mm, 3.92 g], [150 - 75 mm, 23.2 g] and [75 - 38 mm, <1.0 g]; V_{max}(KBr)/ cm^{-1} 3022, 2918, 1597, 1487, 1444, 1358, 1265, 1171, 1068, 1015, 990, 900, 823, 759, 696 and 539.

Example 13: Evaluation of the Scintillant Resins Constructed Using Monomer 5

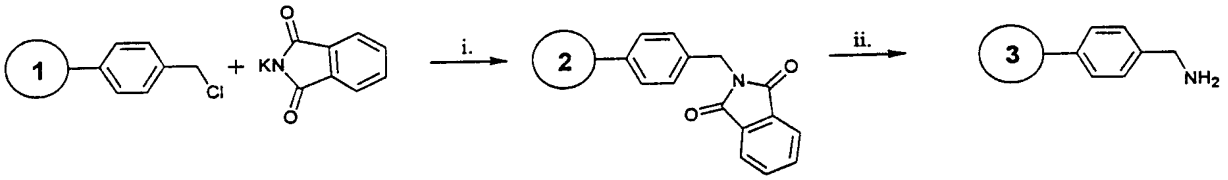
Each scintillant resin constructed using monomer 5 was assessed for scintillation counting efficiency. In two separate control experiments, a gel-type resin and a macroporous resin each of which contained no scintillant were also assessed.

Specifically, 10-100 mg of each resin were weighed into polypropylene scintillation vials. A stock solution of ^{14}C labeled hexadecane in toluene was prepared. A 500 μl aliquot of this stock solution was added to each vial. In each case, it was found that this volume of stock solution was sufficient to fully swell (where appropriate) and cover all of the resin samples. The vials were then monitored in a scintillation counter. The total activity of the stock solution was obtained by counting four 500 μl aliquots in scintillation cocktail, total counts per aliquot = 138761cpm. In addition, 500 μl of the toluene/^{14}C hexadecane stock solution were counted, in the absence of any scintillant, to give a background count

from the radioactivity source alone. The results obtained from scintillation counting the scintillant gel-type resins are presented in Fig.2 and the results obtained for the macroporous scintillant resins are presented in Fig.3. From these studies it was found that the optimum scintillant gel-type resin was the resin that incorporated 2% oxazole and that the optimum scintillant macroporous resin was the resin that incorporated 1% oxazole.

Example 14: Chemical Derivatisation of the Optimum Scintillant Gel-type Resin

Synthesis of aminomethyl-polystyrene scintillant gel-type resin 3



Reagents: i. Dimethylacetamide, 80°C. ii. Aqueous methylamine, dioxane, RT.

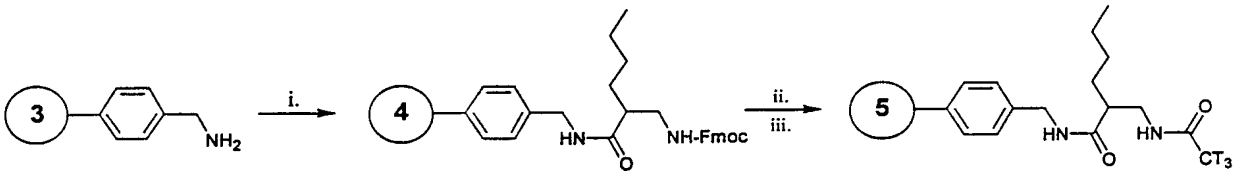
Scheme 7

The optimum scintillant gel-type resin, namely, gel-type 1% cross linked chloromethylpolystrene-2% oxazole resin 1, (150 - 75 μm), (2.00 g, theoretically 1 mmol) and potassium phthalimide (0.74 g, 4.00 mmol) were placed under an atmosphere of nitrogen. Dimethylacetamide (50 cm^3) was added and the mixture was stirred at 80°C overnight. The resin was collected by filtration and washed *in situ* with dimethylformamide, dichloromethane and methanol to give the phthalimide derived scintillant resin 2 (2.21 g); V_{max}(KBr)/ cm^{-1} 3024, 2920, 1713, 1599, 1490, 1446, 1388, 1180, 1023, 938, 755, 694 and 530.

The phthalimidomethyl scintillant resin 2 (2.21 g) was suspended

in dioxane (50 cm^3). Aqueous methylamine (40% wt., 5.00 cm^3) was added and the mixture shaken for 3 days at room temperature. The resin was collected by filtration and washed *in situ* with dioxane:20% aqueous hydrochloric acid (4:1), dioxane:water (4:1), 10% triethylamine in *N,N*-dimethylformamide, *N,N*-dimethylformamide, dichloromethane and methanol to give scintillant aminomethyl resin as a white solid 3 (1.82 g); V_{max}(KBr)/ cm^{-1} 3020, 2916, 1597, 1488, 1446, 1363, 1181, 1156, 1068, 1026, 901, 829, 756, 694 and 533.

Synthesis of norleucine containing scintillant gel-type resin 4 and tritiated resin 5



Reagents i. Fmoc-NorLeu-OH, PyBOPä, HOBt, $^{i}Pr_2EtN$, DMA;
ii. Piperidine, DMF
iii. Tritiated acetic anhydride, DMAP, pyridine,DCM.

Scheme 8

Scintillant aminomethyl gel-type - 2% oxazole resin 3 (1.00 g) was shaken with Fmoc-NorLeu-OH (0.71 g, 2.00 mmol), hydroxybenzotriazole (0.31 g, 2.00 mmol), PyBOP© (1.04 g, 2.00 mmol) and di-*iso*-propylethylamine (706 μl; 0.52 g, 4.00 mmol) in dimethylformamide (10 cm^3) for 1 hour. After this time, the resin was collected by filtration and washed *in situ* with 1-methyl-2-pyrrolidinone, dichloromethane and methanol. After drying briefly, the resin was subjected to a capping reaction using a dimethylacetamide solution (5.00 cm^3) containing acetic anhydride (0.3 M), pyridine (0.3 M) and

dimethylaminopyridine (0.10 mol%) for 1 hour at room temperature. After the capping reaction, the resin was collected by filtration and washed *in situ* with 1-methyl-2-pyrrolidinone, dichloromethane and methanol. The loading of the resin was assessed using a standard Fmoc deprotection protocol (piperidine in DMF, NOVA Biochem 97/98 Catalogue page S37) which gave a loading of 0.78 mmol/g.

The capped Norleucine derived resin 4 was treated with piperidine to remove the Fmoc group (see resin loading protocol above) and the resultant free amino terminal of the Norleucine was radiolabeled by reaction with tritiated acetic anhydride using conditions identical to those employed in example 10, see page 41. The resultant tritiated resin 5 was then assessed for scintillation counting efficiency. As a control experiment, a resin that had undergone exactly the same reaction sequence but that did not contain scintillant was assessed in analogous fashion. The counts for each resin were measured i) dry *i.e.* in the absence of any solvent, ii) swollen after the addition of 0.5cm^3 of toluene and iii) in Ultima Gold to give the total counts possible. The results of these assays are presented below in Table 1.

Resin	Counts per minute per mg of dry resin / cpm	Counts per minute per mg of swollen resin / cpm	Counts per minute per mg of resin in Ultima Gold / cpm
5	120	201	290
Blank control	0	0	185

Table 1 Scintillation counting results for the tritium labeled Norleucine derived scintillant gel-type resin.

The counts per minute obtained for the scintillant containing gel-type resin indicate that the scintillating efficiency of this resin is 40% as efficient as the commercial scintillation cocktail, Ultima Gold, when counted dry and that this efficiency increases to 69 % when the resin is counted in a swollen state.

Example 15 : Synthesis of an Aqueous Compatible Scintillant Resin

Vinylpolystyrene-1%-oxazole-macroporous resin using 2-ethylhexanol as a porogen

Using the standard suspension polymerisation reaction conditions and resin purification procedures, styrene (15.0 cm^3 , 13.6 g, 133 mmol), 80 % divinylbenzene (35.0 cm^3 , 32.0 g, 194 mmol, {ethylstyrene, 48.5 mmol}), (4'-vinyl)-4-benzyl-2,5-diphenyloxazole (monomer 5) (1.27 g, 3.77 mmol), azobisisobutyronitrile (0.32g, 1.95 mmol) and 2-ethylhexanol (50 cm^3) gave scintillant containing resin beads in the size range 150 - 75 μm (21.7 g); V_{max}(KBr)/ cm^{-1} 2925, 1650, 1507, 1456, 903 and 797. These beads were then assessed for scintillation counting in aqueous media.

Aqueous Assay of Vinylpolystyrene-1%-oxazole-macroporous resin

An aqueous solution of ^{33}P was prepared by the addition of approximately 2 μl of a ^{33}P labelled ATPase enzyme to water (12.0 cm^3). The activity of 500 μl of this solution in scintillation cocktail was 54006 cpm. 10 - 100 mg samples of vinylpolystyrene-1%-scintillant-macroporous resin were weighed into polypropylene scintillation vials and 500 μl of the ^{33}P solution was added. In order to wet the beads fully, 100 μl of ethanol and a solution of 1% triton X-100 in water (1.00 cm^3) were also added to each vial. The vials were then monitored in a scintillation counter. The results obtained are presented in Fig.4 and indicate that it is possible to use these supports in aqueous media as well as in organic solvents.

It will be appreciated that this aqueous compatible scintillant resin can be funtionalised by including a chemically functionalised monomer in the polymer mixture such as in the resins of Example 12. A macroporous aqueous compatible resin including chemically functionalised groups is particularly suitable for combinational chemistry applications.

C L A I M S :

1. A support for a chemical application having at least one chemically reactive site, the support formed from at least one scintillant monomer by polymerisation or copolymerisation of the monomer.

2. A support according to claim 1 formed from at least one additional, chemically functionalised, monomer.

3. A support according to claim 1 or 2 formed from at least one additional, inert, monomer.

4. A support according to claim 1, 2 or 3 for use in solid phase synthetic chemistry or solid phase combinatorial chemistry.

5. A support according to any of claims 1 to 4 having polymer cross-linking.

6. A support according to any of claims 1 to 5 in the form of a bead, with a diameter in the range 0.5-500 micrometers.

7. A bead according to claim 6 which has a porous or macroporous structure.

8. A support according to any of claims 1 to 4 or 7 in the form of a gel type polymer.

9. A support for a chemical reaction according to any of claims 1 to 3 or 5 to 8.

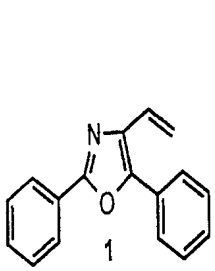
10. A support according to any of claims 1 to 9 in which the

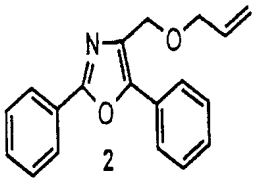
scintillant monomer comprises a molecule of structure R-Y; in which R is a scintillant group and Y is a group comprising a polymerisable moiety.

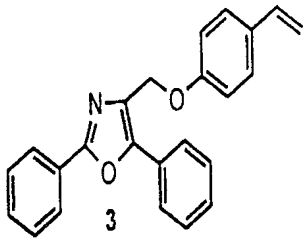
11. A support according to claim 10 in which R is 2,5-diphenyloxazole.

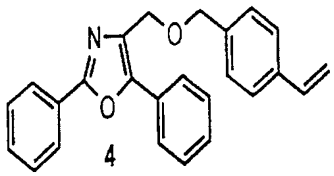
12. A support according to claim 10 or 11 in which Y is a substituted or unsubstituted aliphatic or aromatic group or an ether.

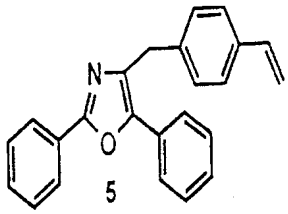
13. A support according to any of claims 1 to 9 in which the scintillant monomer is one of the following :











14. An assay incorporating the steps of :

[a] providing a support for a chemical application having at least one chemically reactive site, the support formed from at least one scintillant monomer by polymerisation or copolymerisation of the monomer ;

[b] mixing the support with a molecule comprising an activating group and a site which may react with the reactive site on the support; and

[c] measuring the scintillation produced by the scintillant monomer.

15. A method for determining how many chemically reactive sites there are on or within a scintillant solid support, incorporating the steps of:

[a] providing a known amount of a support for a chemical application having at least one chemically reactive site, the support formed from at least one scintillant monomer by polymerisation or copolymerisation of the monomer;

[b] mixing the support with a molecule comprising a site which may bind with the chemically reactive site on the support and an activating group; and

[c] measuring the scintillation produced by the

scintillant monomer.

16. A method of monitoring the progress of a chemical reaction comprising the steps of:

[a] providing an activating group and a known amount of a support for a chemical application having at least one chemically reactive site, the support formed from at least one scintillant monomer by polymerisation or copolymerisation of the monomer, the chemically reactive site being bound to a reactant molecule comprising a site which binds or reacts with the chemically reactive site on the support;

[b] measuring the scintillation produced by the scintillant monomer;

[c] subjecting the support to reaction conditions whereby the activating group is removed from the reactant molecule such that the activating group is removed from the support; and

[d] measuring the scintillation produced by the scintillant monomer.

17. A method of monitoring the progress of a chemical reaction comprising the steps of:

[a] providing a known amount of support for a chemical application having at least one chemically reactive site, the support formed from at least one scintillant monomer by polymerisation or copolymerisation of the monomer;

[b] mixing the support with a molecule comprising a site which may bind with the chemically reactive site on the support and an activating group; and

[c] measuring the scintillation produced by the scintillant monomer.

18. A support for a chemical application comprising a polymer matrix with a scintillant moiety covalently bonded into the polymer matrix and at least one chemically reactive site.

19. A support according to claim 18 in which the scintillant moiety is distributed substantially uniformly throughout the polymer matrix.

20. A support according to claim 18 or 19 in which the chemically reactive site is an integral part of, and distributed substantially uniformly throughout, the polymer matrix.

21. A support according to claim 18 or 19 in which the chemically reactive site is formed at the surface of the matrix as a layer.

22. A support according to any of claims 18-21 in the form of a bead, with a diameter in the range 0.5 micrometers to 1 centimeter.

23. A support according to any of claims 18-22 which has porous or macroporous structure.

24. A support according to any of claims 18-21 disposed as a layer on a substrate.

25. A support according to any of claims 18-24 formed by polymerisation of a monomer comprising the scintillant moiety.

26. An assay incorporating the steps of :

[a] providing a support for a chemical application comprising a polymer matrix having at least one scintillant moiety and at least one chemically reactive site, the scintillant moiety being covalently bonded into the polymer matrix;

[b] mixing the support with a molecule comprising an activating group and a site which may react with the reactive site on the support; and

[c] measuring the scintillation produced by the scintillant moiety.

27. A support for use in solid phase synthetic chemistry

comprising a polymer matrix with a scintillant moiety covalently bonded into the polymer matrix and a chemically reactive site.

28. A support for use in combinatorial chemistry comprising a polymer matrix with a scintillant moiety covalently bonded into the polymer matrix and a chemically reactive site.

29. A support according to claim 27 or 28 in which the scintillant moiety is distributed substantially uniformly throughout the polymer matrix.

30. A support according to claim 27, 28 or 29 in the form of a bead, with a diameter in the range 0.5-500 micrometers.

31. A support according to claim 27, 28, 29 or 30, in which the polymer matrix has more than 20% polymer- cross linking.

32. A support according to claim 27, 28, 29, 30 or 31 having porous or macroporous structure.

33. A support according to claim 27, 28 or 29 in the form of a gel type polymer.

34. A method for determining how many chemically reactive sites there are on or within a scintillant solid support, incorporating the steps of:

[a] providing a known amount of support for a chemical reaction comprising a polymer matrix having at least

one scintillant moiety and at least one chemically reactive site, the scintillant moiety being covalently bonded into the polymer matrix;

[b] mixing the support with a molecule comprising a site which may bind with the chemically reactive site on the support and an activating group; and

[c] measuring the scintillation produced by the scintillant moiety.

35. A method of monitoring the progress of a chemical reaction comprising the steps of:

[a] providing an activating group and a known amount of support for a chemical reaction comprising a polymer matrix having at least one scintillant moiety and at least one chemically reactive site, the scintillant moiety being covalently bonded into the polymer matrix, the chemically reactive site being bound to a reactant molecule comprising a site which binds or reacts with the chemically reactive site on the support;

[b] measuring the scintillation produced by the scintillant moiety;

[c] subjecting the support to reaction conditions whereby the activating group is removed from the reactant

molecule such that the activating group is removed fromthe support; and

[d] measuring the scintillation produced by the scintillant moiety.

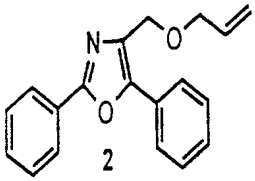
36. A method of monitoring the progress of a chemical reaction comprising the steps of:

[a] providing a known amount of support for a chemical reaction comprising a polymer matrix having at least one scintillant moiety and at least one chemically reactive site, the scintillant moiety being covalently bonded into the polymer matrix;

[b] mixing the support with a molecule comprising a site which may bind with the chemically reactive site on the support and an activating group; and

[c] measuring the scintillation produced by the scintillant moiety.

37. A scintillant monomer of one of the following structures:



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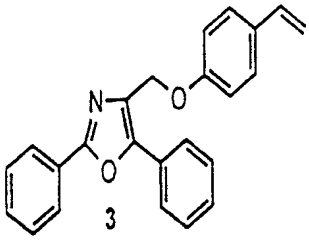

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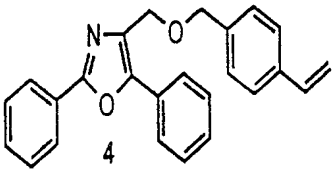

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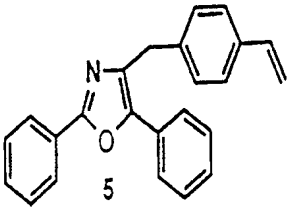

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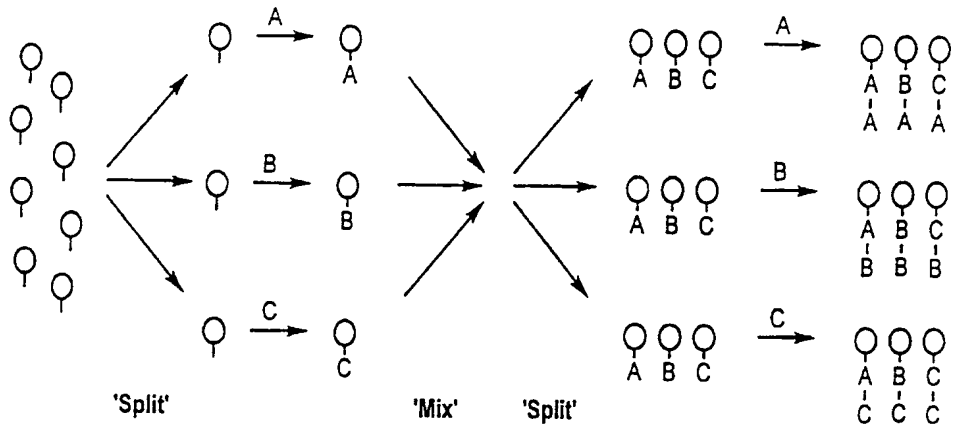
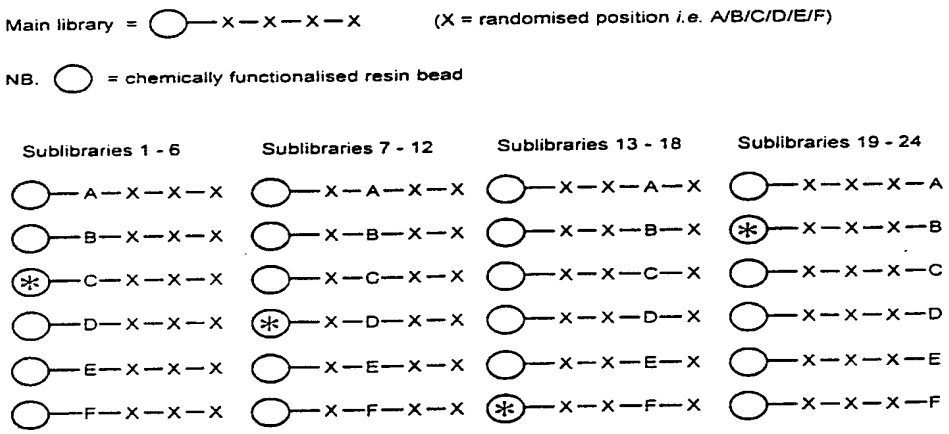


where ⚲ represents a chemically functionalised resin bead.

NB. After these two synthetic steps, every possible dimeric combination of A, B and C on the resin beads has been formed.

FIGURE 1A



In an assay of each set of six sublibraries, the sublibrary marked with an * is the most active.

The identity of the best binder may be deduced to be C-D-F-B

FIGURE 1B

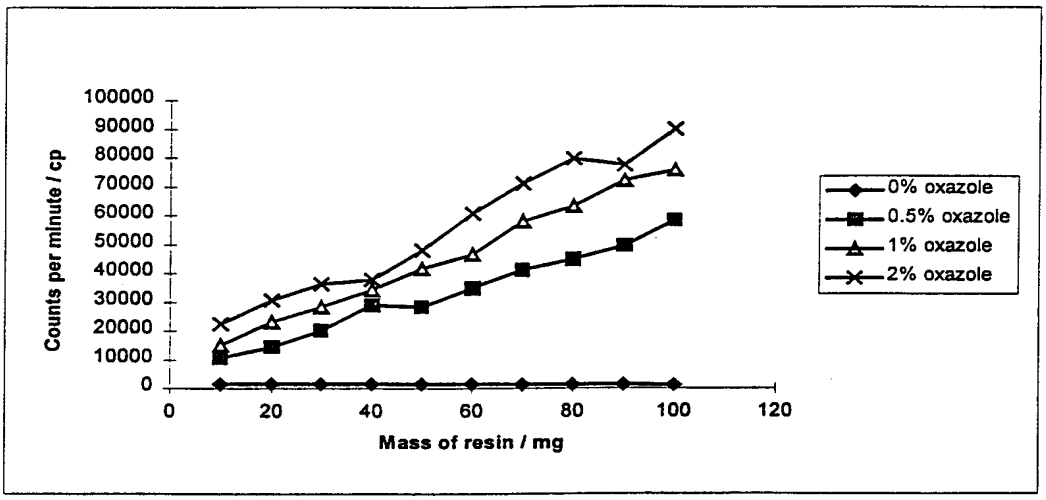

Counts per minute / cp
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0
20
40
60
80
100
120
Mass of resin / mg
0% oxazole
0.5% oxazole
1% oxazole
2% oxazole

Figure 2

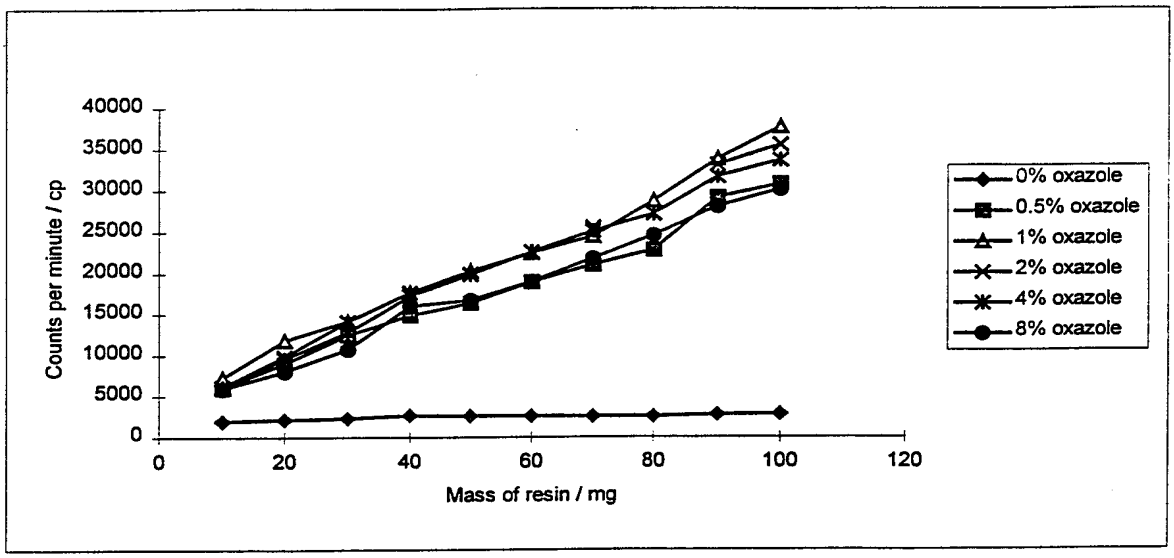

40000
35000
30000
25000
20000
15000
10000
5000
0
Counts per minute / cp
0
20
40
60
80
100
120
Mass of resin / mg
0% oxazole
0.5% oxazole
1% oxazole
2% oxazole
4% oxazole
8% oxazole

Figure 3

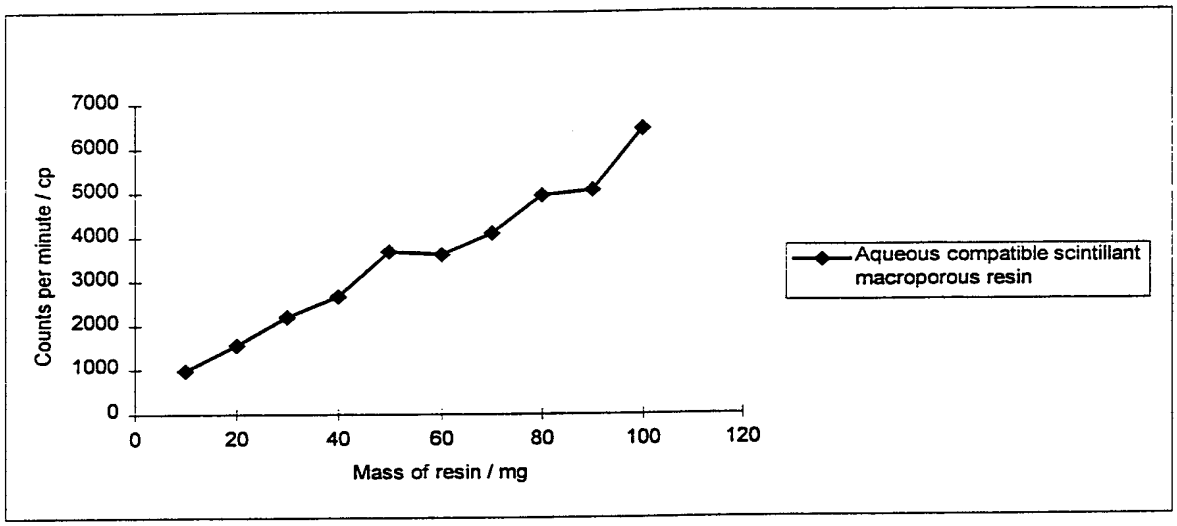

7000
6000
5000
4000
3000
2000
1000
0
Counts per minute / cp
0
20
40
60
80
100
120
Mass of resin / mg
Aqueous compatible scintillant macroporous resin

Figure 4

INTERNATIONAL SEARCH REPORT

Inta. .onal Application No
PCT/GB 99/03296

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C08F212/08 C08F246/00 G01N33/58 G01N33/545

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C08F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 26413 A (AMERSHAM INT PLC ;COOK NEIL DAVID (GB)) 24 November 1994 (1994-11-24) page 13, line 10 - line 12; claims 1,3,12 ---	1,4,8, 11,13
A	DATABASE WPI Section Ch, Week 198722 Derwent Publications Ltd., London, GB; Class A96, AN 1987-152421 XP002132047 & JP 62 047555 A (HOFFMANN-LA ROCHE AG), 2 March 1987 (1987-03-02) cited in the application abstract --- -/--	1,6,14, 18,22

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 March 2000	20/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Engel, S

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03296

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 108, no. 20, 1988 Columbus, Ohio, US; abstract no. 168329, "ecximer photophysics of macromolecular scintillators" XP002132045 abstract -& BIRCH ET AL.: ACS SYMP. SER., vol. 358, pages 170-185, XP002132131 ISSN: 0097-6156 page 172, paragraph 4	1,13,36
A,P	WO 99 09415 A (JESSOP ROBERT A ;NYCOMED AMERSHAM PLC (GB)) 25 February 1999 (1999-02-25) page 4, line 17 -page 6, line 6	1,14,18, 21,22,36
A	CHEMICAL ABSTRACTS, vol. 69, no. 20, 1968 Columbus, Ohio, US; abstract no. 78048, GRIGOR'EVA ET AL.: "Plastic scintillators based on copolymers of styrene with vinyl derivatives of 2,5-diaryloxazoles..." XP002132046 abstract & ZH. PRIKL. SPEKTROSK., vol. 8, no. 5, 1968, pages 884-887, USSR	1,3, 10-12

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/GB 99/03296

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		ES 2072847 T	01-08-1995
		FI 950154 A	13-01-1995
		JP 7509405 T	19-10-1995
		US 5665562 A	09-09-1997
		US 5989854 A	23-11-1999
JP 62047555 A	02-03-1987	NONE	
WO 9909415 A	25-02-1999	AU 8742998 A	08-03-1999